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Research Article

SOLUBILITY ENHANCEMENT OF RITONAVIR: CO-CRYSTALLIZATION

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ABSTRACT

The main objective of this work is to explore co-crystallization approach for increasing solubility of an antiretroviral drug, Ritonavir (RTN). In this study, different co-formers with different functional groups like carboxylic acid and acid amide were tried in ratio of 1:1, 1:2 and 1:3 (RTN : Co-former) using neat grinding method. Co-formers used were citric acid (CIT) and adipic acid (ADP). The co-crystals formed were characterized by melting point determination, Fourier-transform infrared (FTIR), Differential Scanning Calorimetry (DSC), X-ray diffraction (XRD) and solubility studies. Co-crystals of drug with CIT and ADP showed the improved dissolution profile when compared to the pure RTN. Melting point, DSC, FTIR spectra of co-crystals were different than pure drug and co-formers indicating their interaction. XRD patterns of co-crystals were not completely amorphous but less intense compared to drug alone.

KEYWORDS: *Ritonavir, Co-crystallization, Adipic acid, Citric acid, Solubility enhancement.*

INTRODUCTION

Ritonavir (RTN) is a BCS class II drug with low solubility high permeability resulting in less bioavailability. In-order to increase solubility and bioavailability of ritonavir, co-crystallization method was approached using adipic acid and citric acid as coformers. It is antiretroviral drug; the protease inhibitor class used to treat HIV infection and AIDS.

Solubility is of great importance in pharmaceutical systems since a drug must be dissolved before it is absorbed. Solubility is generally defined as thermodynamic equilibrium of a solute between two phases. When the drug absorption process is limited by dissolution, enhancing solubility can increase dissolution rate and in this way improve bioavailability. In addition to the dissolution rate, the ability to dissolve the therapeutic dose of the drug and the selection of solubilization techniques are critical for product development. The oral bioavailability of a drug, which is by definition the degree at which its active form is made available at the site of action after oral administration, is mainly dependent on the solubility of the drug in the gastrointestinal tract, its permeability through the intestinal wall and on effects of the pharmaceutical formulation [1]. According to the biopharmaceutics classification system (BCS), class II drugs are most suitable for bioavailability enhancement through pharmaceutical formulation due to their good permeability which is only limited by their dissolution rate [2].

Cocrystals can be defined in several ways [3, 4]. A restrictive definition is that cocrystals are structurally homogeneous crystalline materials containing two or more components present in definite stoichiometric amounts [5]. The cocrystal components are discrete neutral molecular reactants which are solids at ambient temperature. Based on this definition of cocrystals, a pharmaceutical cocrystal means a cocrystal with one of the components as an Active Pharmaceutical Ingredient (API) and the other components are called coformers. From the definition, it is clearly shown that an API hydrate is not a cocrystal; however, a solid-state API hydrate can crystallize with a solid coformer to form a cocrystal [6]. Currently cocrystal approach is a method of great interest for the pharmaceutical industry. Apart from offering potential improvements in solubility, dissolution rate, bioavailability and physical stability, pharmaceutical cocrystals can enhance other essential properties of the APIs such as flow ability, chemical stability, compressibility and hygroscopicity [7].

The primary difference between cocrystals and salts is that in salts there is a proton transfer from the acidic to the basic functionality of the crystallization partner, or vice versa if applicable, whereas in cocrystals no such transfer takes place. On the other hand, the main difference between cocrystals and solvates is the physical state of the individual pure components: if one component is in liquid- state at room temperature, the crystals are designated as solvates; if both components are solids at room temperature, the crystals are designated as cocrystals. Till now, pharmaceutical cocrystals are emerging as

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promising materials in drug discovery and development, particularly in modifying drug properties such as dissolution rate, solubility, bioavailability, stability, hygroscopicity, compressibility and flow ability.

MATERIALS AND METHODS

All the excipients and active pharmaceutical ingredient (API) used for the development of ritonavir co-crystals were listed below:

Table No. 1: List of materials used

S. No.	Materials	Supplied by
1	Ritonavir	Raks Pharma Pvt. Ltd. Visakhapatnam, Andhra Pradesh
2	Adipic acid	Hi-media, Mumbai
3	Citric acid	Finar, Ahmedabad

Pre-formulation Studies:

1) **Solubility study:** The solubility of ritonavir, adipic acid and citric acid was determined.

2) **Melting point determination:** Melting point of ritonavir, citric acid and adipic acid was determined by open capillary method.

3) **Identification test:** Identification of ritonavir, adipic acid and citric acid was carried out by FTIR spectrophotometer.

4) **Construction of calibration curve:** Primary stock solution of 1mg/ml was prepared. For this accurately weighed amount(100mg) of ritonavir was transferred into 100ml volumetric flask and diluted with 0.1 N HCl to make up the volume to 100ml.Secondary stock was prepared by diluting 10 ml of primary stock solution to 100ml with 0.1 N HCl to obtain 100µg/ml solution. From this secondary stock,10µg/ml working stock solution was prepared by transferring 10ml of secondary stock into100 ml volumetric flask and diluting with0.1 N HCl up to the mark. Solution of 2, 4, 6,8,10 ml was taken separately from working stock and make up to10 ml with 0.1 N HCl to produce 2, 4, 6,8,10 µg/ml respectively. The absorbance of sample was measured at 244.4 nm using a double beam UV-visible spectrophotometer. The linear regression equations were obtained to fit the data of unknown concentration into them and to know the drug concentration in the unknown samples and also to know the drug release.

Preparation of ritonavir cocrystals:

1) **Preparation of cocrystals using adipic acid as coformer (Dry grinding method):** Ritonavir and adipic acid were taken in the ratio 1:1 (A1) and 1:2 (A2)and triturated separately in a mortar with pestle for about 60 minutes in clockwise direction until a homogenous mixture is obtained and transferred to an air-tight container and stored at room temperature.

2) **Preparation of cocrystals using citric acid as coformer (Dry grinding method):** Ritonavir and citric acid were taken in ratio 1:1 (C1), 1:2 (C2) and 1:3 (C3)triturated in a mortar and pestle for about 60 minutes in clockwise direction until a homogenous mixture is obtained and transferred to an air-tight container and stored at room temperature.

Evaluation studies:

a) **In vitro dissolution studies:** Dissolution studies of the pure drug ritonavir and co-crystal preparations were conducted using type II apparatus (Electro lab, dissolution tester, TDT-08L), with paddle type at the rotation sped of 50rpm in 900ml of 0.1N HCl which is used as dissolution medium. The temperature was maintained at 37±0.5°C using temperature controller (Electrolab, ETC-11). The dissolution process was done for about 90minutes under sink conditions. The samples of volume 5ml were collected at the time interval of 15minutes and replaced with 5ml 0.1N HCl. The samples were analyzed in UV-Visible spectrophotometer (Systronics, India). The dissolution profile was examined and evaluated for amount of drug released.

b) **Determination of drug content:** For determining the drug content in the preparation (cocrystal) for the formulation with better dissolution profile, equivalent amount of preparation (cocrystals) containing 10mg of ritonavir was taken and dissolved in 10ml of methanol, shaken for 10-15 minutes and the volume was made up to100ml using SGF. The samples were analyzed using UV-Visible spectrophotometer (Systronics, India) and λ_{max} of 244nm.All the observations were done in triplicate and the average and standard deviation was calculated.

c) **Determination of Melting point:** The melting point of the co-crystals (with better drug release profile)was measured in melting point apparatus by taking the samples in the capillary tubes.

d) **Fourier-transform infrared spectroscopy (FTIR):** IR spectroscopy was performed for ritonavir, adipic aid, citric acid and the cocrystal preparation(formulation with better drug release profile) using a FTIR (Shimadzu, IRTracer-100) spectrophotometer which was employed to characterize the possible interactions between the drug and coformer in solid-state. Samples were prepared using KBr pellet method. Samples of about 2mg were lightly ground and mixed with 200mg IR grade dry potassium bromide and then compressed at 10 tonnes in a hydraulic press to form discs. Spectroscopy was done at room temperature (35±5°C).

e) **Differential scanning calorimetry:** The molecular state of the drug in cocrystal formulation was evaluated by performing DSC analysis of pure drug, citric acid and their corresponding co-crystal preparation (formulation with better drug release profile). The DSC curves of the samples were obtained by differential scanning calorimeter (Mettler DSC 823e, Mettler-Toledo, Germany). Average sample weight of 5±2mg was heated in thermatically sealed aluminum pan over a temperature range of 50°C to 300°C under a constant nitrogen gas flow of 30ml/min at a heating rate of 10°C/min. The instrument was calibrated

with indium(calibration standard, purity > 99.9%) for melting point and heat of fusion.

f) Powder X-ray diffraction (P-XRD): Samples containing pure ritonavir, coformer (citric acid) and the cocrystal preparations (formulation with better drug release profile) of ritonavir: citric acid (1:2) was sent for powder X-ray analysis. The diffractogram was obtained using X-ray diffractometry (Bruker AXS D8 Advance) at Karthikeya drugs and pharmaceuticals Pvt, Ltd. The X-ray source used was Cu at a wavelength of 1.5406 Å. Temperature was maintained at -170° to 450°C using low-temperature chamber (Anton Paar, TTK 450). Based on the diffraction pattern, the Relative Degree of Crystallinity (RDC) of the cocrystal preparations ritonavir citric acid was calculated.

RESULTS AND DISCUSSIONS

1) Solubility:

Ritonavir: Freely soluble in methanol and ethanol, soluble in isopropanol.

Adipic acid: Slightly soluble in water, freely soluble in ethanol.

Citric acid: Very soluble in water, freely soluble in ethanol and soluble in ether.

2) Construction of calibration curve of ritonavir in 0.1N HCl using UV-Visible spectrophotometry:

The standard graph ofritonavir hydrochloride has shown good linearity with regression co-efficient value 0.997 in 0.1N HCl which suggest that it obeys the Beer-Lambert's law as seen in figure 5.

Table No. 2: Calibration curve of ritonavir 0.1N HCl

Concentration(µg/ml)	Absorbance(nm)
0	0
10	0.1371
20	0.2751
30	0.3918
40	0.5326
50	0.6748
60	0.8106
70	0.9339

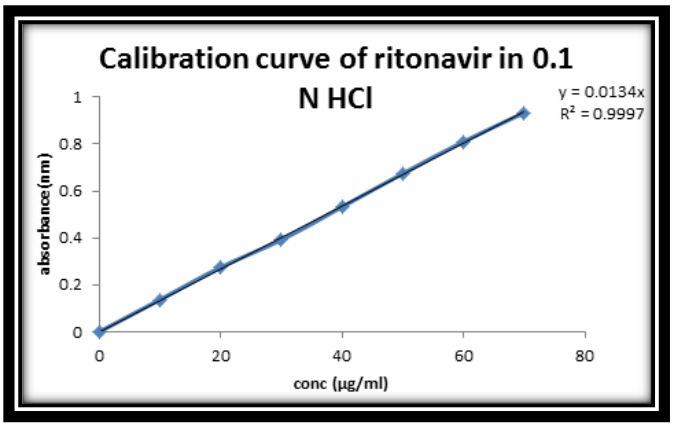

Fig. 1: Calibration curve of ritonavir in 0.1N HCl

3) Drug Dissolution study: The Dissolution study was conducted for cocrystal preparations containing adipic acid and citric acid using 0.1N HCl using USP type II apparatus at 37±0.5°C for 90mins. The dissolution profile of the cocrystals that contain citric acid showed 100% drug release in 60 minutes for C2 and pure drug showed 82% drug release in 90 minutes (table 3 and fig.2). This indicates that C2 cocrystals show faster drug release compare to the C1, C3 and pure drug. Whereas, cocrystal formulations that contain adipic acid showed 98% drug release of the drug in 90 minutes for A2 (1:2 ratio) when compared to A1 and pure drug (table 3 and fig.2). The drug release profiles of pure drug and all the formulations were captured below:

Table No. 3: In vitro drug release of pure drug in 0.1N HCl

Time(min)	% Drug release					
	Drug	C1	C2	C3	A1	A2
0	0	0	0	0	0	0
15	28	6	34	4	10	32
30	35	13	52	10	15	38
45	50	17	80	13	23	53
60	68	28	100	20	35	85
90	82	36	104	30	43	98

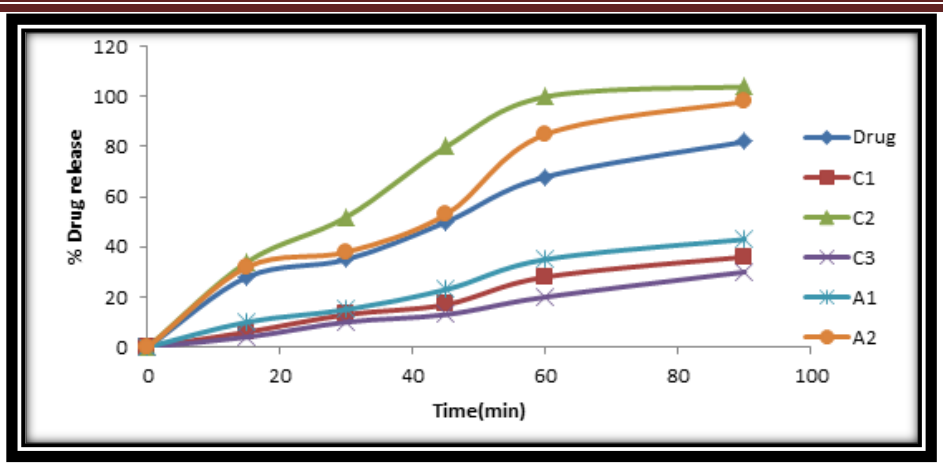

Fig. 2: The dissolution profile of pure drug and ritonavir-adipic acid (A1-1:1, A2-1:2) and ritonavir-citric acid (C1-1:1, C2-1:2, C3-1:3) cocrystals in 0.1N HCl. Each point represents the mean±SD (n=3).

4) **Drug content determination:** The drug content was determined for the cocrystals formulations (C2, formulation with better dissolution profile) in triplicates. The values were 98±0.85%given in table 4. The values indicate good content uniformity for the cocrystal formulations. These results indicate that neat dry grinding method can be used to develop cocrystals of ritonavir.

Table No. 4: Drug content of cocrystal preparations

Cocrystal formulations	Percent drug content
C2: ritonavir – citric acid (1:2)	98±0.85

5) **Melting Point:** As co-crystallization leads to change in physico-chemical properties, melting point of prepared cocrystal (C2), pure drug (ritonavir) and coformer (citric acid and adipic acid) were noted. It was found that the melting point of the co-crystals showed a significant deviation with respect to the melting point of pure drug and the individual co-formers indicating their interaction. This test was used as a preliminary test for confirmation of co-crystal formation for further characterization.

6) **Fourier Transform Infrared (FTIR) Studies:** FTIR is an excellent technique to give an insight into the kind of study the chemical and physical structure changes in the molecular structure ofa substance. As shown in Fig.3, the FTIR spectrum of RTN showed presence of C=O amide peak at 1697.41 cm^{-1}C=O ester peak at 1750.04cm^{-1} -NH- stretch at 3379.40cm^{-1}, amide –NH-bend at 1527.67cm^{-1}. There is a shift in the functional groups present in the spectra of co-crystal (C2) (fig. 6) compared to that of drug, the C=O amide peak is shifted from 1697.41cm^{-1} to 1635.69$cm^{-1}$of ritonavir citric acid cocrystals, C=O ester peak shifted to lower frequency from 1750.04 to 1716$cm^{-1}$of ritonavir citric acid cocrystal, -NH-stretch of ritonavir citric acid is found at 3426$cm^{-1}$when compared to pure drug, amide –NH- bend shifted from 1527.67cm^{-1} to 1514.17cm^{-1}. There is a shift in the FTIR frequency of functional groups present in the cocrystals compared to that of drug, showing the presence of the formation of new bonds.

Table No. 5: Melting points of formulation (C2), pure drug and coformers

S. No	Sample	Melting point (°C)
1	C2	100-105
2	Ritonavir (pure drug)	122-124
3	Citric acid (coformer)	149-153
4	Adipic acid (coformer)	151-154

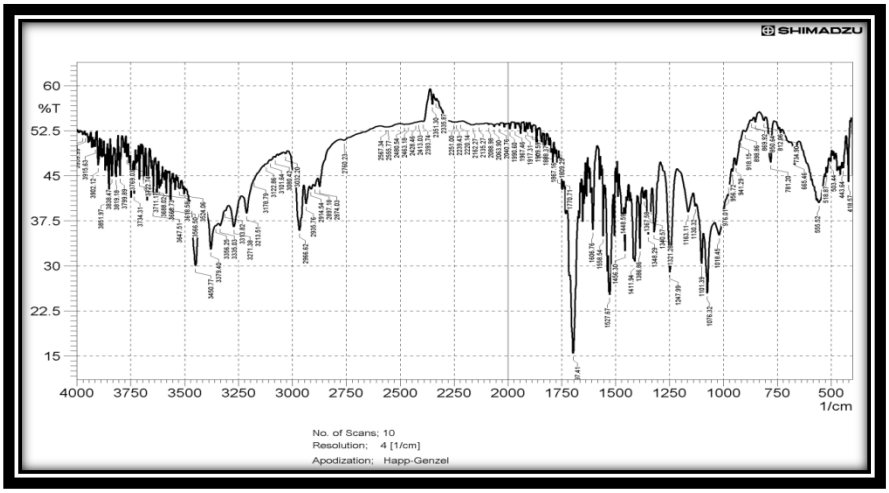

Fig. 3: FTIR spectra of pure ritonavir

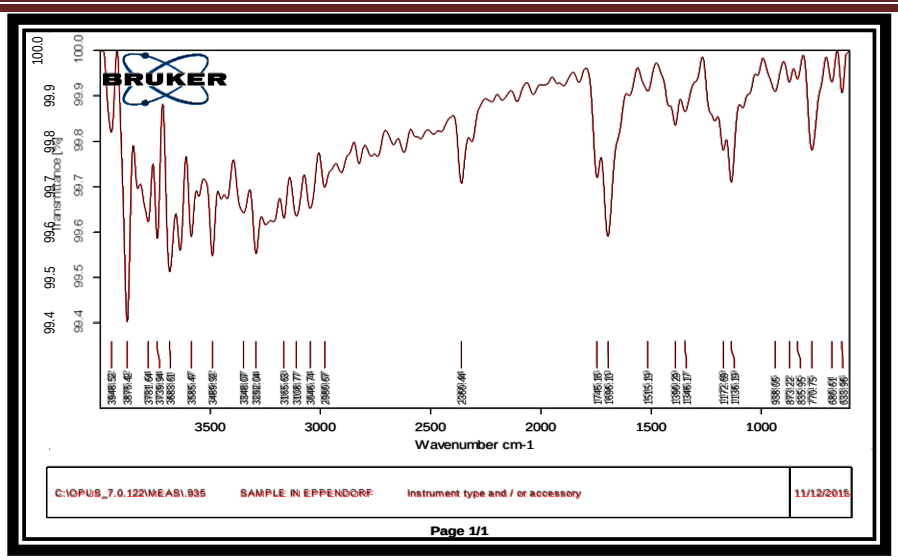

Fig. 4: FTIR spectra of citric acid

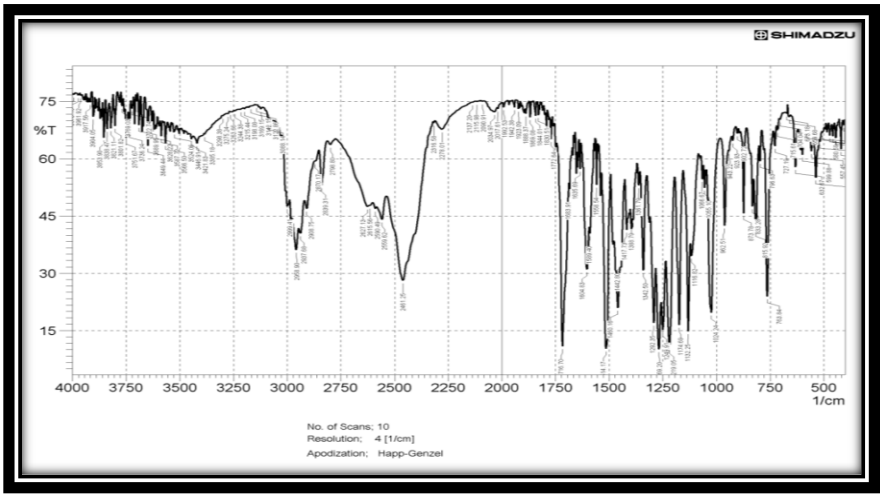

Fig. 5: FTIR spectra of C2 formulation (ritonavir, citric acid in 1:2 ratios)

7) Differential scanning calorimetry (DSC): The thermotropic behavior and physical state of drug in cocrystals was evaluated by performing DSC analysis. From the DSC thermograms (fig.7, 8 and 9), it was observed that the thermograms of the co-crystals were different in pattern and intensity as compared to RTN and co-formers which indicates their interaction. This shift in the melting point is due to the change in crystal lattice of the RTN in presence of co-former, forming a relatively different crystal lattice in co-crystals.

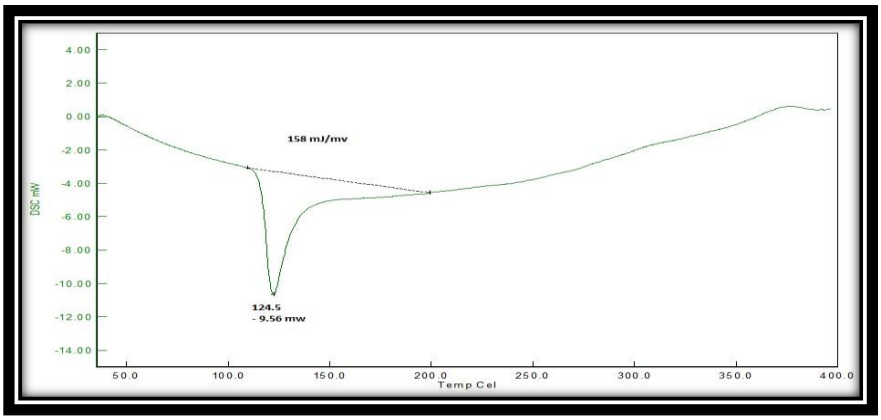

Fig. 6: DSC thermogram of ritonavir

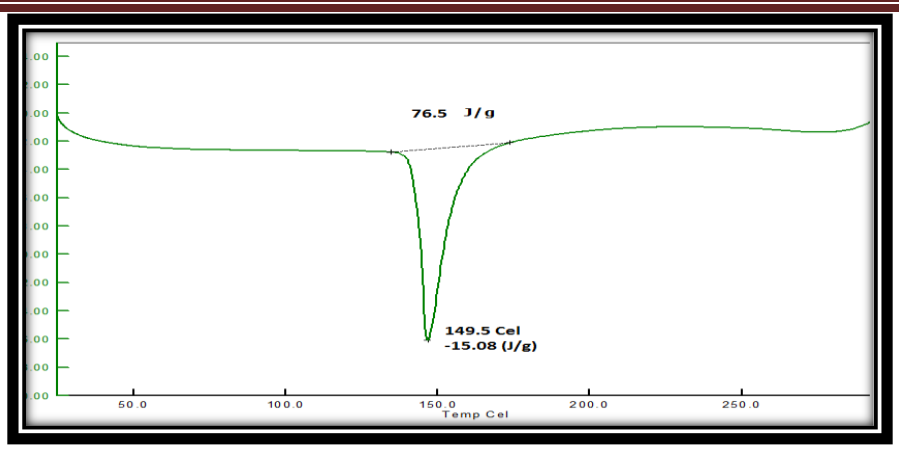

Fig. 7: DSC thermogram of citric acid

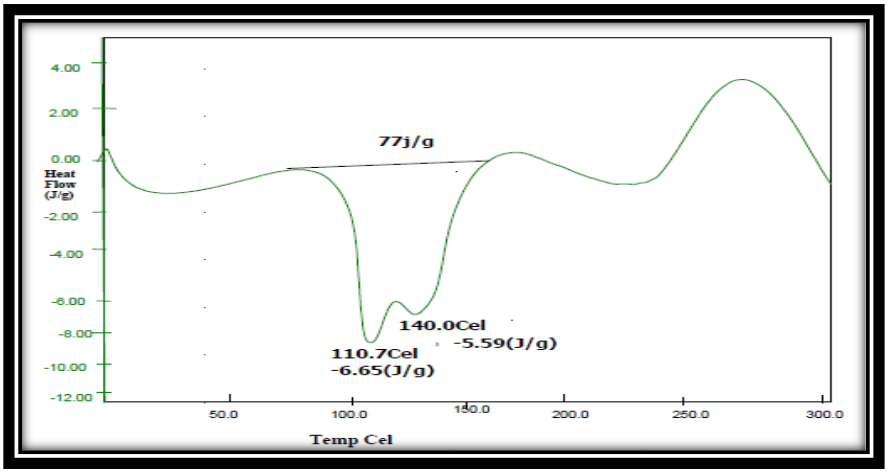

Fig. 8: DSC thermogram of ritonavir-citric acid (C2)

8) Powder X-Ray Diffraction: The diffraction pattern of the pure ritonavir (fig.10) showed high intensity peaks at 2-theta values at 18.14°, 19.16°, 21.56°, 23.62°, 25.98° and 28.23°. The diffraction pattern of citric acid showed a singular peak at 44.08°. Two other peaks were observed at 37.85° and 64.43°.

The diffraction pattern of the cocrystal formulation C2 (fig.12) showed a sharp peak at 17.05°, 26.10°, 29.02°, 29.75° and 77.55° which was unseen in diffraction patterns both the components (pure ritonavir and citric acid) of the complex. The peak obtained at 23.22° which was closer to the peak observed in pure ritonavir, but it was found to be reduced in intensity when compared with pure ritonavir. The other intense peaks of ritonavir were reduced indicating a decrease in the degree of crystallinity.

The relative degree of crystallinity (RDC) was calculated for the C2 formulation which showed a decrease in the crystallinity in cocrystal formulations when compared to the pure ritonavir. This corresponds to the decrease in the intensity of the ritonavir peaks in cocrystal formulations in the diffraction pattern (table 6).

Table No. 6: Relative degree of crystallinity of the cocrystal formulation C2 at different 2θ positions

Type of system	18.14 Pos		21.56 Pos		23.62 Pos		25.98 Pos		28.23 Pos	
	Height (cts)	RDC	Height (cts)	RDC	Height (cts)	RDC	Height (cts)	RDC	Height (cts)	RDC
Pure drug	213	-	381	-	505	-	222	-	211	-
C2 (formulation)	68	0.31	175	0.45	389	0.77	316	1.42	186	0.77

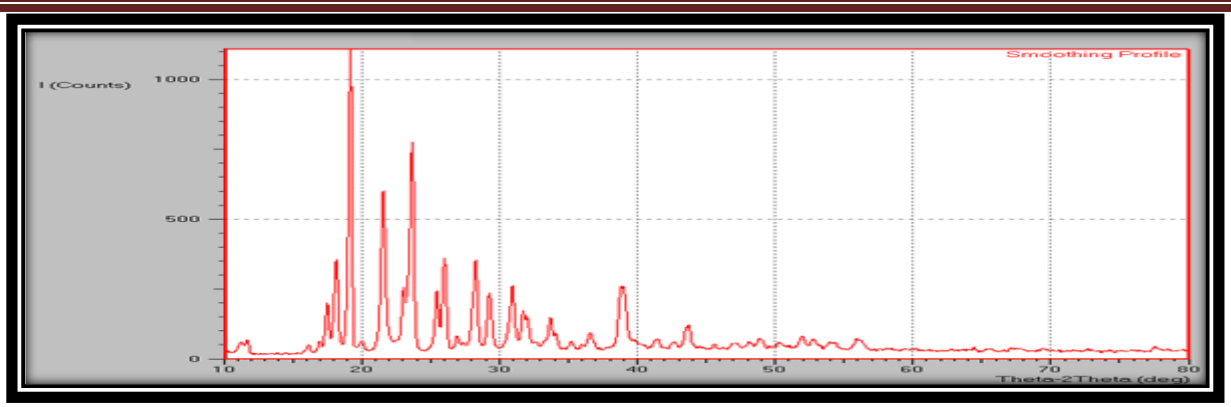

Fig. 9: Diffractogram of pure ritonavir

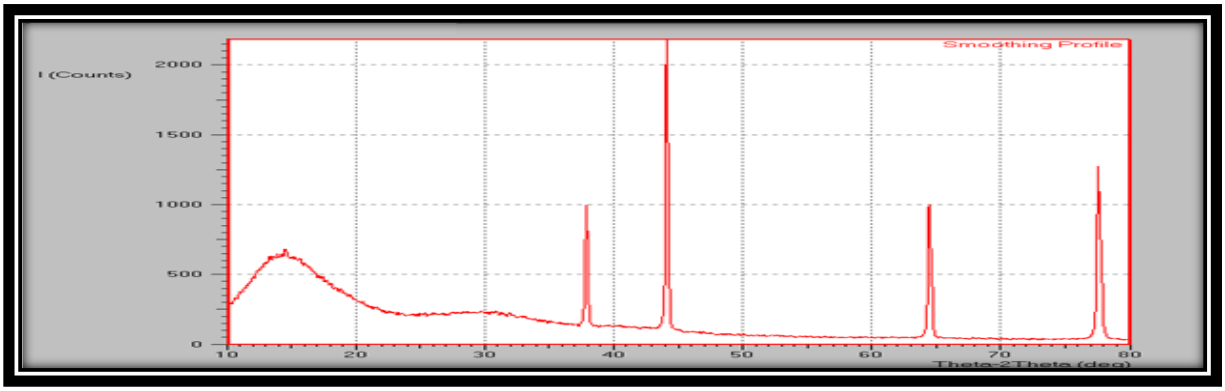

Fig. 10: Diffractogram of citric acid

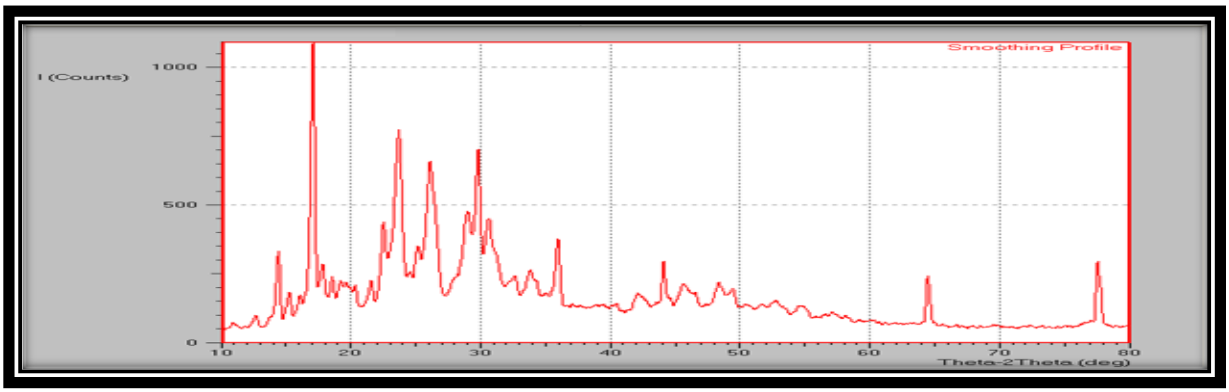

Fig. 11: Diffractogram of C2 (ritonavir–citric acid 1:2)

CONCLUSION

The co-crystals were prepared successfully using different coformers by neat dry grinding method. These co-crystals were characterized by melting point, FTIR, DSC and XRD. These studies indicated formation of new crystal phases due to physical and or chemical interactions between API and co-former. For RTN, dicarboxylic acid co-crystals showed better solubility and dissolution. In vitro dissolution studies were performed, and it shows that there was 100% drug release in 60minutes for cocrystal formation and pure ritonavir shows 82% release at 90minutes. The initial dissolution rate of cocrystals was considerably faster compare to pure ritonavir. Improved dissolution efficiency and relative dissolution rate of cocrystals than pure drug reveals those cocrystals as suitable carriers for improved solubility and dissolution rate of pure ritonavir.

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