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STUDIES ON ENHANCEMENT OF SOLUBILITY AND DISSOLUTION RATE OF RITONAVIR EMPLOYING β-CYCLODEXTRIN, SOLUPLUS AND PVPK30

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ABSTRACT

Ritonavir, a widely prescribed anti retroviral drug belongs to class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. Its oral absorption is dissolution rate limited and it requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. The objective of the study is to enhance the solubility and dissolution rate of ritonavir by cyclodextrin complexation along with Soluplus and PVP K30 and to evaluate the individual main effects and combined (or interaction) effects of β cyclodextrin (βCD), surfactant (Soluplus) and PVP K30 on the solubility and dissolution rate of ritonavir in a series of 2^3 factorial experiments. The effects of βCD, Soluplus and PVP K30 alone on the solubility of ritonavir were evaluated by phase solubility studies. The solubility of ritonavir in eight selected fluids containing βCD, Soluplus and PVP K30 as per 2^3 factorial study was determined. Solid inclusion complexes of ritonavir-βCD were prepared with and without Soluplus and PVP K30 by kneading method as per 2^3-factorial design and were evaluated. The aqueous solubility of ritonavir was increased linearly as a function of the concentration of βCD as well as Soluplus and PVP K30.The phase solubility diagram of ritonavir –β CD complexes is of type A_L. Increase in solubility of ritonavir was due to the formation of a 1:1 M complex in solution with βCD with a stability constant (Kc) value of 325.97 M -1.The individual and combined effects of βCD, Soluplus and PVP K30 in enhancing the solubility and dissolution rate of ritonavir were highly significant ($P < 0.01$). βCD alone gave a 2.24 fold increase in the solubility of ritonavir. Combination of βCD with

Soluplus or PVP K30 resulted in a much higher enhancement in the solubility of ritonavir, 3.97 fold with βCD- Soluplus and 3.44 fold with βCD- PVP K30. There was no further increase in the solubility of ritonavir when βCD is combined with both Soluplus and PVP K 30.The dissolution of ritonavir was rapid and higher in the case of ritonavir- βCD complex systems when compared to ritonavir pure drug. βCD alone gave a 1.54 fold increase in the dissolution rate of (K1) of ritonavir. When βCD is combined with Soluplus or PVP K30 the dissolution rate (K1) was significantly enhanced to 7.67 fold and 5.67 fold with β CD – soluplus and β CD – PVP complexes. There was no further increase in the dissolution rate with ritonavir – β CD - Soluplus - PVP K 30 quaternary complexes. Hence complexation of ritonavir with β CD – Soluplus and β CD – PVP K 30 is recommended to enhance the solubility and dissolution rate of ritonavir, a BCS Class II drug.

KEYWORDS: Ritonavir, β Cyclodextrin, Soluplus, PVP K30, Solubility, Dissolution rate, Factorial Study.

INTRODUCTION

Ritonavir, a widely prescribed protease inhibitor drug belongs to class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. It is practically insoluble in water and aqueous fluids. As such its oral absorption is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Several techniques[1] such as micronization, cyclodextrin complexation, use of surfactants and solubilizers, solid dispersion in water soluble and dispersible carriers, use of salts, prodrugs and polymorphs which exhibit high solubility, micro emulsions and self emulsifying micro and nano disperse systems have been used to enhance the solubility, dissolution rate and bioavailability of poorly soluble drugs. Among the various approaches complexation with cyclodextrins has gained good acceptance in recent years in industry for enhancing the solubility and dissolution rate of poorly soluble drugs. Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity which can accommodate a variety of lipophilic drugs. As a consequence of inclusion process many physico-chemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably affected.[2,3] Cyclodextrins have been receiving increasing application in pharmaceutical formulation in recent years due to their approval by various regulatory agencies.[4,5] Soluplus is a polymeric solubiliser with an amphiphilic chemical nature, which was particularly developed for solid solutions.[6] Soluplus is polyvinyl

caprolactam – polyvinyl acetate – polyethylene glycol graft co- polymer. Soluplus increased the solubility and enhanced the bioavailability of actives in solid solutions. Itraconazole and fenofibrate showed significant increase in the bioavailability with Soluplus.[6] The solubility and dissolution rate of valsartan was effectively enhanced by using Soluplus in the form of solid dispersions.[7] Poly vinyl pyrrolidone (PVP K 30) is also reported.[8,9] to enhance the solubility and dissolution rate of poorly soluble drugs.

Though cyclodextrin complexation and use of surfactants and PVP for enhancing the solubility and dissolution rate of poorly soluble drugs have been investigated individually, no reports are available on their combined use in enhancing the solubility and dissolution rate. In the present investigation the individual main effects and combined (or interaction) effects of β cyclodextrin (βCD), surfactant (Soluplus) and PVP K30 on the solubility and dissolution rate of ritonavir, a BCS class II drug were evaluated in a 2^3 factorial study.

EXPERIMENTAL

MATERIALS

Ritonavir was a gift sample from M/s. Eisai Pharmatechnology and Manufacturing Pvt. Ltd., Visakhapatnam. β Cyclodextrin was gift sample from M/s. Cerestar Inc., USA. Soluplus was a gift sample from BASF, the chemical company, Hyderabad. Methanol (Qualigens) and poly vinyl pyrrolidone (PVP K30) were procured from commercial sources. All other materials used were of pharmacopoeial grade.

METHODS

Estimation of Ritonavir

A UV Spectrophotometric method based on the measurement of absorbance at 240 nm in 0.1 N HCl was used for the estimation of ritonavir. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 0-10 µg/ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variance were found to be 0.85% and 1.20 % respectively. No interference by the excipients used in the study was observed.

Phase Solubility Study

The effects of βCD, Soluplus and PVP K30 alone on the solubility of ritonavir were evaluated by phase solubility studies as per Higuchi and Connors.[10] Excess drug (50 mg) was added to 15 ml of each fluid taken in a 25 ml stoppered conical flask and the mixtures

were shaken for 24 h at room temperature (28±1°C) on Rotary Flask Shaker. After 24 h of shaking, 2 ml aliquots were withdrawn at 2 h interval and filtered immediately using a 0.45 µ disk filter. The filtered samples were diluted suitably and assayed for ritonavir by measuring absorbance at 240 nm. Shaking was continued until two consecutive estimations are the same. The solubility experiments were replicated for three times each (n=3).

Preparation of Ritonavir - βCD Complexes

Solid inclusion complexes of ritonavir – βCD - Soluplus - PVP K30 were prepared as per 2^3 – factorial study by kneading method. Ritonavir, βCD, Soluplus and PVP K30 were triturated in a mortar with a small volume of solvent consisting of a blend of dichloromethane: methanol (1:1). The thick slurry formed was kneaded for 45 min and then dried at 55°C until dry. The dried mass was powdered and sieved to mesh No. 120.

Dissolution Rate Study

The dissolution rate of ritonavir as such and from βCD complexes prepared was studied in 0.1 N HCl using Disso 2000 (Labindia) 8-station dissolution test apparatus with a paddle stirrer at 50 rpm. A temperature 37±1°C was maintained throughout the study. Ritonavir or ritonavir- βCD complex equivalent to 50 mg of ritonavir was used in each test. Samples of dissolution media (5 ml) were withdrawn through a filter (0.45 µ) at different intervals of time, suitable diluted and assayed for ritonavir at 240 nm. The sample of dissolution fluid withdrawn at each time was replaced with fresh fluid. The dissolution experiments were replicated three times each (n=3).

Analysis of Data

Solubility and dissolution data were analyzed by Analysis of Variance (ANOVA) as per 2^3 factorial study.

RESULTS AND DISCUSSION

The objective of the study is to enhance the solubility and dissolution rate of ritonavir by cyclodextrin complexation along with Soluplus and PVP K30 and to evaluate the individual main effects and combined (or interaction) effects of β cyclodextrin (βCD), surfactant (Soluplus) and PVP K30 on the solubility and dissolution rate of ritonavir in a series of 2^3 factorial experiments.

The effects of βCD, Soluplus and PVP K30 on the solubility of ritonavir were initially evaluated by phase solubility studies. The phase solubility diagrams showing the effects of

βCD, Soluplus and PVP K30 and their concentrations on the solubility of ritonavir are shown Figs. 1-3. The aqueous solubility of ritonavir was increased linearly as a function of the concentration of βCD as well as Soluplus and PVP K30. The phase solubility diagram of ritonavir –β CD complexes (Fig.1) can be classified as type A_L according to Higuchi and Connors [10]. Because the straight line had a slope <1, the increase in solubility was due to the formation of a 1:1 M complex in solution with βCD. The apparent stability constant (Kc) was calculated from the slope of the corresponding linear plot of the phase solubility diagram according to the equation, Kc = Slope/So (1-Slope), where So is the solubility of the drug in the absence of βCD. The estimated Kc value of ritonavir - βCD complex was 325.97 M^{-1} indicating that the complexes formed between ritonavir and βCD are quite stable.

The individual main effects and combined (interaction) effects of βCD (Factor A), Soluplus (Factor B) and PVP K30 (Factor C) on the aqueous solubility of ritonavir were evaluated in a series of 2^3-factorial experiments. For this purpose, two levels of βCD (0, 5 mM), two levels of Soluplus (0, 1%) and two levels of PVP K30 (0, 1%) were selected in each case and the corresponding eight treatments involved in the 2^3-factorial study were purified water (1); water containing 5 mM βCD (a); water containing 1% Soluplus (b); water containing 5 mM βCD and 1% Soluplus (ab); water containing 1% PVP K30 (c); water containing 5 mM βCD and 1% PVP K30 (ac); water containing 1% Soluplus and 1% PVP K30 (bc) and water containing 5 mM βCD and 1% of each of Soluplus and PVP K30 (abc).

The solubility of ritonavir in the above mentioned fluids was determined (n=3) and the results are given in Table-1. The solubility data were subjected to Analysis of variance (ANOVA) to find out the significance of main and combined effects of βCD, Soluplus and PVP K30 on the solubility of ritonavir. The results of ANOVA (Table 2) indicated that the individual and combined effects of βCD, Soluplus and PVP K30 in enhancing the solubility of ritonavir were highly significant ($P < 0.01$).

βCD alone gave a 2.24 fold increase in the solubility of ritonavir. Combination of βCD with Soluplus and PVP K30 resulted in a much higher enhancement in the solubility of ritonavir, 3.97 fold with βCD- Soluplus and 3.44 fold with βCD- PVP K30, than with βCD alone. Soluplus also gave an enhancement of 2.58 folds in the solubility of ritonavir. There was no further increase in the solubility of ritonavir when βCD is combined with both Soluplus and PVP K 30. The combination of β CD - Soluplus - PVP K 30 gave an enhancement of 2.80 folds in the solubility of ritonavir. To evaluate the individual and combined effects of βCD,

Soluplus and PVP K30 on the dissolution rate of ritonavir, solid inclusion complexes of ritonavir- βCD were prepared with and without Soluplus and PVP K30 as per 2^3-factorial design. For this purpose two levels of βCD (0 and 1:2 ratio of drug : βCD) and two levels of each of Soluplus and PVP K30 (0 and 1%) were selected and the corresponding eight treatments involved in the 2^3-factorial study were ritonavir pure drug (1); ritonavir- βCD (1:2) inclusion binary complex (a); ritonavir - Soluplus (1%) binary complex (b); ritonavir- βCD (1:2) - Soluplus (1%) ternary complex (ab); ritonavir – PVP K30 (1%) binary complex (c); ritonavir- βCD (1:2) - PVP K30 (1%) ternary complex (ac); ritonavir - Soluplus (1%) - PVP K30 (1%) ternary complex (bc) and ritonavir-βCD (1:2) - Soluplus (1%) - PVP K30 (1%) complex (abc).

The CD complexes were prepared by kneading method. All the solid inclusion complexes of ritonavir- βCD - Soluplus /PVP K30 prepared were found to be fine and free flowing powders. Low coefficient of variation (c.v.) values (< 1.2 %) in the percent drug content indicated uniformity of drug content in each batch of solid inclusion complexes prepared. The dissolution rate of ritonavir alone and from βCD complexes was studied in 0.1 N HCl as prescribed in IP 2010. The dissolution of ritonavir followed first order kinetics with R^2 (coefficient of determination) values above 0.912. Dissolution efficiency (DE_{30}) values were calculated as suggested by Khan.[11] The dissolution parameters are given in Table 3. The dissolution of ritonavir was rapid and higher in the case of ritonavir- βCD complex systems prepared when compared to ritonavir pure drug as such. The dissolution profiles are given in Fig. 4. The dissolution rate (K_1) values were subjected to ANOVA to find out the significance of the main and combined effects of βCD, Soluplus and PVP K30 on the dissolution rate of ritonavir. ANOVA (Table 4) indicated that the individual main effects of βCD, Soluplus and PVP K30 and their combined effects in enhancing the dissolution rate (K_1)and dissolution efficiency (DE_{20}) were highly significant ($P < 0.01$). βCD alone gave a 1.54 fold increase in the dissolution rate of (K_1) of ritonavir. When βCD is combined with Soluplus or PVP K30 the dissolution rate (K_1) was significantly enhanced. A 7.67 and 5.67 fold increase in the dissolution rate (K_1) was observed respectively with ritonavir - βCD – Soluplus and ritonavir - βCD – PVP K30 solid inclusion complexes. There was no further increase in the dissolution rate with ritonavir – β CD - Soluplus - PVP K 30 quaternary complexes. These complexes gave an enhancement of 4.23 folds in the dissolution rate of ritonavir. DE_{30} values were also much higher in the case of βCD solid complexes when compared to ritonavir pure drug. Thus the ternary complexes i.e., ritonavir - βCD - soluplus

and ritonavir - βCD - PVP K 30 gave higher enhancement in the dissolution rate of ritonavir than βCD alone and ritonavir – β CD - Soluplus - PVP K 30 quaternary complexes. Hence complexation of ritonavir with β CD – Soluplus and β CD – PVP K 30 is recommended to enhance the solubility and dissolution rate of ritonavir, a BCS Class II drug.

Table 1: Solubility of Ritonavir in Various Fluids containing βCD, Soluplus and PVP K30 as per 2^3 – Factorial Study

Fluids (Code as per 2^3 – Factorial Experiment)	Solubility (mg/ml) (n=3) ($\bar{x}$ ± s. d.)	Increase in Solubility (Number of Folds)
Distilled water (1)	0.148±0.003	-
Water containing 5 mM βCD (a)	0.331±0.005	2.24
Water containing 1% Soluplus (b)	0.381±0.001	2.58
Water containing 5mM βCD and 1% Soluplus (ab)	0.588±0.006	3.97
Water containing 1% PVP K30 (c)	0.205±0.005	1.38
Water containing 5mM βCD and 1% PVP K30 (ac)	0.510±0.074	3.44
Water containing 1% Soluplus and 1% PVP K30 (bc)	0.240±0.016	1.62
Water containing 5mM βCD, 1% Soluplus and 1% PVP K30 (abc)	0.415±0.005	2.80

Table 2: ANOVA of Solubility Data of Ritonavir in Various Fluids as per 2^3 – Factorial Study (βCD - Soluplus– PVP K30)

Source of variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	23	2.980	0.130	3747.41	P<0.01
Treatments	7	2.978	0.425		
a	1	0.268	0.268	2360.11	P<0.01
b	1	0.071	0.071	624.01	P<0.01
ab	1	0.002	0.002	15.57	P<0.01
c	1	0.002	0.002	15.88	P<0.01
ac	1	0.007	0.007	58.13	P<0.01
bc	1	0.144	0.144	1266.85	P<0.01
abc	1	0.011	0.011	100.76	P<0.01
Error	16	0.00182	0.000114	-	-

$F_{0.01\ (7,\ 16)} = 4.03$; $F_{0.05\ (7,\ 16)} = 2.66$; $F_{0.01\ (1,\ 16)} = 8.53$; $F_{0.05\ (1,\ 16)} = 4.49$

Table 3: Dissolution Parameters of Ritonavir – βCD- Soluplus- PVP K30 Solid Inclusion Complexes Prepared as per 2^3 Factorial Study

R –CD Complex	Composition	PD_{10} (%)	K_1x10^2 min^{-1}	Increase in K_1 (No. of folds)	DE_{30} (%)	Increase in DE_{30} (No. of folds)
RF1	R	15.73	0.64	---	16.55	---
RFa	R-βCD(1:2)	25.90	0.99	1.54	29.91	1.80
RFb	R-Soluplus (1%)	31.53	1.83	2.85	37.14	2.24
RFab	R-βCD (1:2)-Soluplus (1%)	69.30	4.90	7.67	71.32	4.30
RFc	R-PVP (1%)	28.20	1.54	2.40	31.06	1.87
RFac	R- βCD (1:2)--PVP (1%)	53.35	3.62	5.67	57.68	3.48
RFbc	R- Soluplus (1%)-PVP (1%)	59.09	2.15	3.35	58.83	3.55
RFabc	R- βCD (1:2)- Soluplus (1%) - PVP (1%)	37.20	2.70	4.23	47.85	2.89

R: Ritonavir; βCD: β cyclodextrin; PVP K30: Poly Vinyl Pyrrolidone K30.

Table 4: ANOVA of Dissolution Rates of Ritonavir – βCD- Soluplus- PVP K30 Solid Inclusion Complexes Prepared as per 2^3 Factorial Study

Source of variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	23	272.96	11.867		
Treatments	7	272.4	38.91	1111.71	P<0.01
a	1	122.44	122.44	3498.2	P<0.01
b	1	47.18	47.18	1348	P<0.01
ab	1	32.55	32.55	930	P<0.01
c	1	10.07	10.07	28.77	P<0.01
ac	1	0.429	0.429	12.25	P<0.01
bc	1	31.579	31.579	902.2	P<0.01
abc	1	28.145	28.145	804.14	P<0.01
Error	16	0.56	0.035		

$F_{0.01\ (7,\ 16)}$ = 4.03; $F_{0.05\ (7,\ 16)}$ = 2.66; $F_{0.01\ (1,\ 16)}$ = 8.53; $F_{0.05\ (1,\ 16)}$ = 4.49

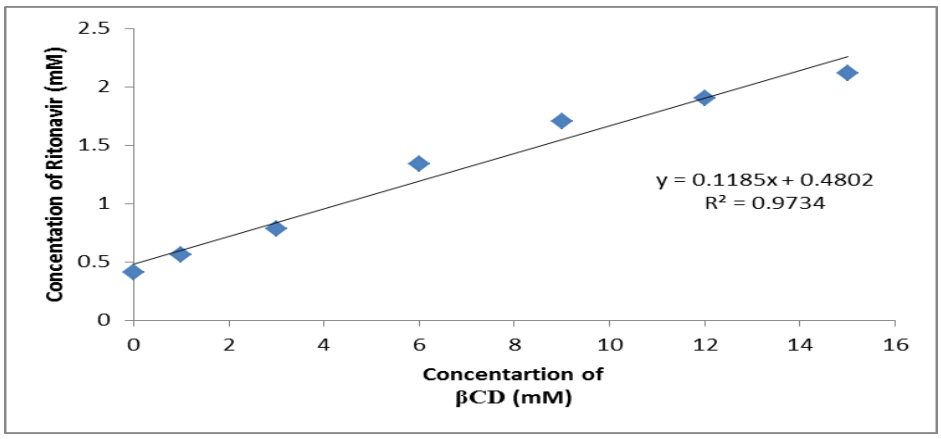

Fig. 1: Phase Solubility Studies - Effect of βCD Concentration on the Solubility of Ritonavir

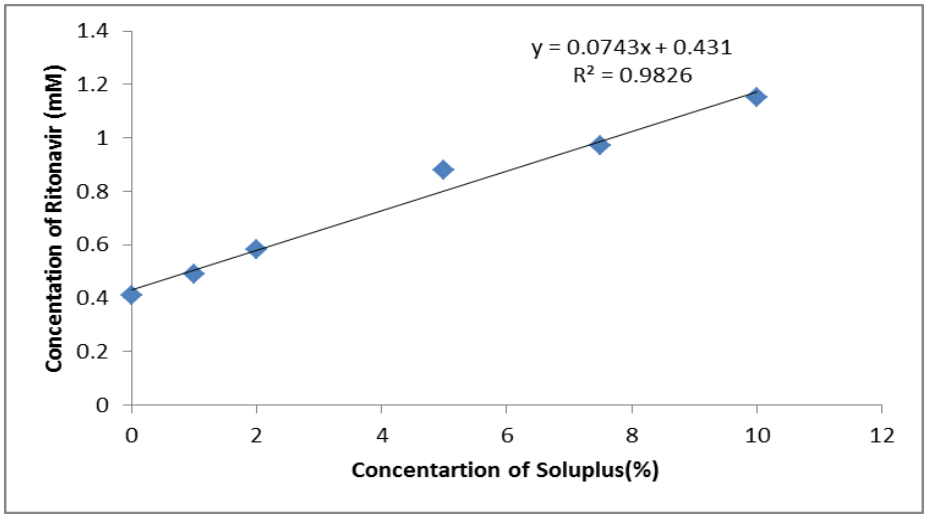

Fig. 2: Effect of Soluplus Concentration on the Solubility of Ritonavir

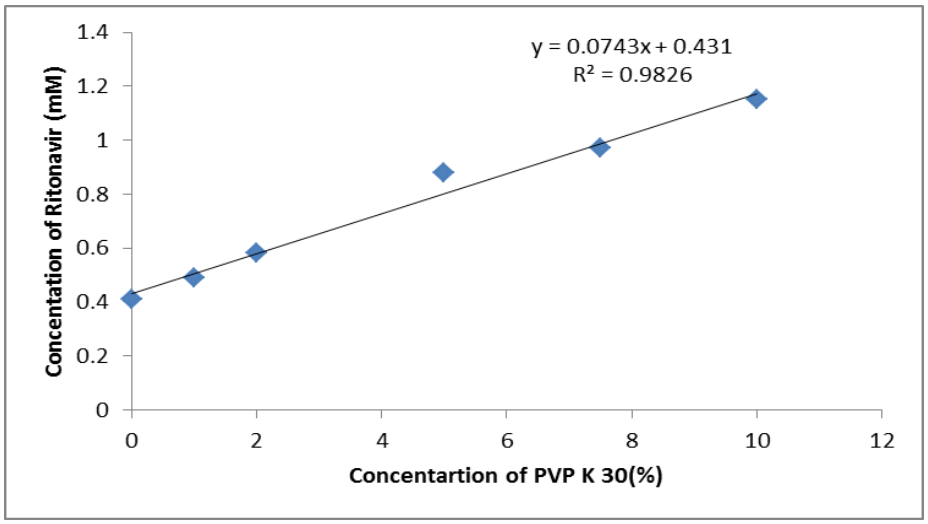

Fig .3: Effect of PVP K30 Concentration on the Solubility of Ritonavir

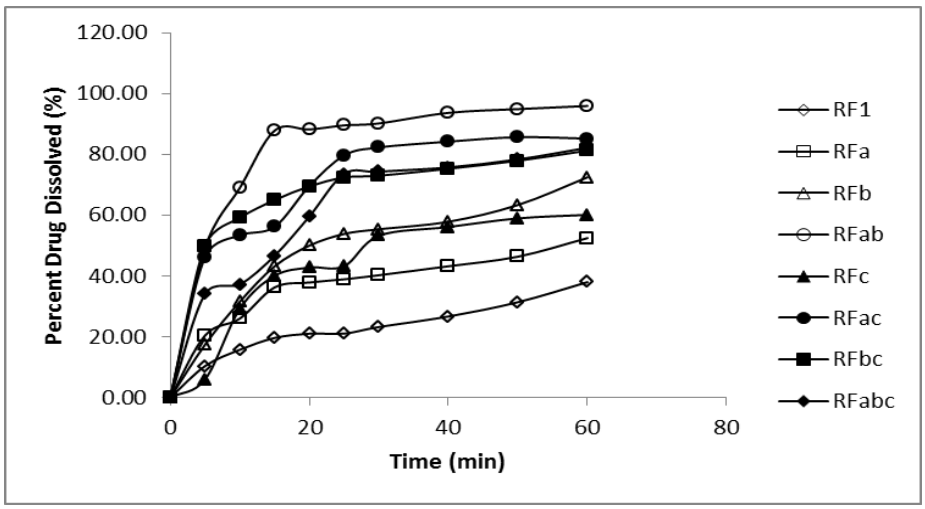

Fig. 4: Dissolution Profiles of Ritonavir – βCD- Soluplus- PVP K30 Solid Inclusion Complexes Prepared as per 2^3 Factorial Study

CONCLUSIONS

1. The aqueous solubility of ritonavir was increased linearly as a function of the concentration of βCD as well as Soluplus and PVP K30.

2. The phase solubility diagram of ritonavir –β CD complexes is of type A_L. Increase in solubility of ritonavir was due to the formation of a 1:1 M complex in solution with βCD with a stability constant (Kc) value of 325.97 M^{-1}.

3. The individual and combined effects of βCD, Soluplus and PVP K30 in enhancing the solubility and dissolution rate of ritonavir were highly significant ($P < 0.01$).

4. βCD alone gave a 2.24 fold increase in the solubility of ritonavir. Combination of βCD with Soluplus or PVP K30 resulted in a much higher enhancement in the solubility of ritonavir, 3.97 fold with βCD- Soluplus and 3.44 fold with βCD- PVP K30. There was no further increase in the solubility of ritonavir when βCD is combined with both Soluplus and PVP K 30.

5. The dissolution of ritonavir was rapid and higher in the case of ritonavir- βCD complex systems when compared to ritonavir pure drug. βCD alone gave a 1.54 fold increase in the dissolution rate of (K_1) of ritonavir. When βCD is combined with Soluplus or PVP K30 the dissolution rate (K_1) was significantly enhanced to 7.67 fold and 5.67 fold with β CD – soluplus and β CD – PVP complexes.. There was no further increase in the dissolution rate with ritonavir – β CD - Soluplus - PVP K 30 quaternary complexes.

6. Hence complexation of ritonavir with β CD – Soluplus and β CD – PVP K 30 is recommended to enhance the solubility and dissolution rate of ritonavir, a BCS Class II drug.

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