ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Enhancement of Solubility and Dissolution Rate of Ritonavir by B Cyclodextrin and Solutol HS 15 – A Factorial Study

KPR Chowdary, Annamma Devi GS* and CH Swapna

AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530003.

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. It is practically in soluble in water and aqueous fluids. As such 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 present study is to enhance the solubility and dissolution rate of ritonavir by the use of β cyclodextrin (βCD) and Solutol HS15 (non ionic surfactant). The individual main effects and combined (or interaction) effect of βCD (Factor A) and Solutol HS15 (Factor B) in enhancing the solubility and dissolution rate of ritonavir were evaluated in a 2^2 factorial study. The solubility of ritonavir in water and water containing selected combinations of the two factors as per 2^2 factorial design was determined. The individual and combined effects of βCD and Solutol HS15 in enhancing the solubility of ritonavir were highly significant ($P < 0.01$). βCD and Solutol HS15 alone gave a 4.66 and 13.57 fold increase in the solubility of ritonavir respectively. Combination of βCD with Solutol HS15 resulted in a much higher enhancement in the solubility of ritonavir, 15.92 fold. Solid inclusion complexes of ritonavir- βCD were prepared with and without Solutol HS 15 as per 2^2-factorial design by kneading method and were evaluated. The individual main effect of Solutol HS15 (Factor B) and combined (interaction) effect of βCD and Solutol HS15 (Factor AB) in enhancing the dissolution rate (K_1) were highly significant ($P < 0.01$), whereas the individual effect of βCD (Factor A) was not significant ($P > 0.05$). βCD alone gave a 7.81 fold increase in the dissolution rate of (K_1) of ritonavir and in combination with Solutol HS15 it gave a 11.79 fold increase in the dissolution rate (K_1) of ritonavir. Solutol HS 15 alone gave higher enhancement in the dissolution rate (15.90 fold) and dissolution efficiency (4.1 fold) of ritonavir. Hence Solutol HS15 alone or a combination of βCD and Solutol HS15 is recommended to enhance the solubility, dissolution rate and dissolution efficiency of ritonavir, a poorly soluble BCS class II drug.

Key Words: Ritonavir, Solubility, Dissolution Rate, β Cyclodextrin, Solutol HS15.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

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. It is practically in soluble in water and aqueous fluids. As such its oral absorption is dissolution rate limited and it requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. Several conventional methods such as micronization, chemical modification, use of surfactants and solubilizers, solid dispersion and a few new emerging technologies such as cyclodextrin complexation, mucoadhesive microspheres, nanoparticles, nanosuspensions, micro emulsion and self-emulsifying systems are available to enhance the solubility, dissolution rate and bioavailability of poorly soluble BCS Class II drugs [1]. 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, 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].

Surfactants increase the solubility of lipophilic water-insoluble drugs by micellar solubilization. Solutol HS15, a non ionic surfactant consists of polyglycol mono- and di-esters of 12-hydroxystearic acid with about 30% polyethylene glycol. Solutol HS15 has been shown to be safe in various animal toxicity models. Solutol HS15 has been approved in Canada and Argentina in marketed injectable drug products. Solutol HS15 has been used as an excellent solubilizer for liquid-filled capsules [6]. It is also reported as a carrier for solid dispersions of nifedipine for enhancing its dissolution rate [7].

Though cyclodextrin complexation and use of Solutol HS15 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 of poorly soluble drugs. The objective of the present study is to enhance the solubility and dissolution rate of ritonavir by the use of β cyclodextrin (βCD) and Solutol HS15. The individual main effects and combined (or interaction) effect of βCD and Solutol HS15 in enhancing the solubility and dissolution rate of ritonavir were evaluated in a 2^2 factorial study.

MATERIALS AND METHODS

Materials

Ritonavir was a gift sample from M/s. Eisai Pharmatechnology and Manufacturing Pvt. Ltd., Visakhapatham. β Cyclodextrin was gift sample from M/s. Cerestar Inc., USA. Methanol (Qualigens), Solutol HS15 were procured from commercial sources. All other materials used were of pharmacopoeial grade.

Methods

Estimation of Ritonavir

An UV Spectrophotometric method based on the measurement of absorbance at 210 nm in 0.1 N hydrochloric acid 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.

Solubility Determination

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^{o}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 210 nm. Shaking was continued until two consecutive estimations are the same. The solubility experiments were replicated for four times each (n=4).

Preparation of Ritonavir - βCD Complexes

Solid inclusion complexes of ritonavir – βCD – Solutol HS15 were prepared as per 2^2 – factorial study by kneading method. Ritonavir, βCD and Solutol HS15 were triturated in a mortar with a small volume of solvent consisting of a blend of water: methanol (1:1). The thick slurry formed was kneaded for 45 min and then dried at 55^{o}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 900 ml 0.1 N hydrochloric acid using Disso 2000 (Labindia) 8-station dissolution test apparatus with a paddle stirrer at 50 rpm. A temperature 37±1^{o}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 210 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 2^2 factorial study.

ISSN: 0975-8585

RESULTS AND DISCUSSION

The individual main effects and combined (interaction) effects of βCD (Factor A) and Solutol HS15 (Factor B) on the aqueous solubility of ritonavir were evaluated in a 2^2-factorial experiment. For this purpose, two levels of βCD (0, 5 mM) and two levels of Solutol HS15 (0, 2%) were selected and the corresponding four treatments involved in the 2^2-factorial study were purified water (1); water containing 5 mM βCD (a); water containing 2% Solutol HS15 (b) and water containing 5 mM βCD and 2% Solutol HS15 (ab).

The solubility of ritonavir in the above mentioned fluids was determined (n=4) and the results are given in Table 1.

Table 1: Solubility of Ritonavir in Various Fluids as per 2^2 - Factorial Study

Fluids (Code as per 2^2 - Factorial Experiment)	Solubility (mg/100 ml) (n=4) $(\bar{x})$ (s.d)	Increase in Solubility (Number of Folds)
Distilled water (1)	2.56±0.211	--
Water containing 5 mM βCD (a)	11.92±0.99	4.66
Water containing 2% Solutol HS 15 (b)	34.73±0.89	13.57
Water containing 5 mM βCD and 2% Solutol HS 15 (ab)	40.76±1.52	15.92

The solubility data were subjected to Analysis of Variance (ANOVA) to find out the significance of main and combined effects of βCD, and Solutol HS15 on the solubility of ritonavir.

Table 2: ANOVA of Solubility Data

Source of Variation	D F	S.S	MSS (SS/DF)	F - Ratio
Total	15	3985.7	265.72	
Treatment	3	3973.4	1324.46	1288.72
Error	12	12.3	1.03	
Factor A	1	237.8	237.78	231.36
Factor B	1	3724.7	3724.66	3624.15
Factor AB	1	10.9	10.96	10.66

$F_{0.05}(3, 12) = 3.49$; $F_{0.05}(1, 12) = 4.75$; $F_{0.01}(3, 12) = 5.95$; $F_{0.01}(1, 12) = 9.33$

The results of ANOVA (Table 2) indicated that the individual and combined effects of βCD and Solutol HS15 in enhancing the solubility of ritonavir were highly significant ($P < 0.01$). βCD and Solutol HS15 alone gave a 4.66 and 13.57 fold increase in the solubility of ritonavir respectively. Combination of βCD with Solutol HS15 resulted in a much higher enhancement in the solubility of ritonavir, 15.92 fold. Solutol HS15 alone gave higher enhancement in the solubility of ritonavir than βCD alone.

To evaluate the individual and combined effects of βCD and Solutol HS15 on the dissolution rate of ritonavir, solid inclusion complexes of ritonavir- βCD were prepared with and without Solutol HS15 as per 2^2-factorial design. For this purpose two levels of βCD (0 and 1:2 ratio of drug : βCD) and two levels of Solutol HS15 (0 and 2%) were selected and the

ISSN: 0975-8585

corresponding four treatments involved in the 2^2-factorial study were ritonavir pure drug (1); ritonavir- βCD (1:2) inclusion complex (a); ritonavir - Solutol HS15 (2%) binary complex (b) and ritonavir- βCD (1:2) - Solutol HS15 (2%) inclusion complex (ab).

The CD complexes were prepared by kneading method. All the solid inclusion complexes of ritonavir- βCD - Solutol HS15 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 hydrochloric acid as prescribed in IP 2010. The dissolution profiles are given in Fig 1.

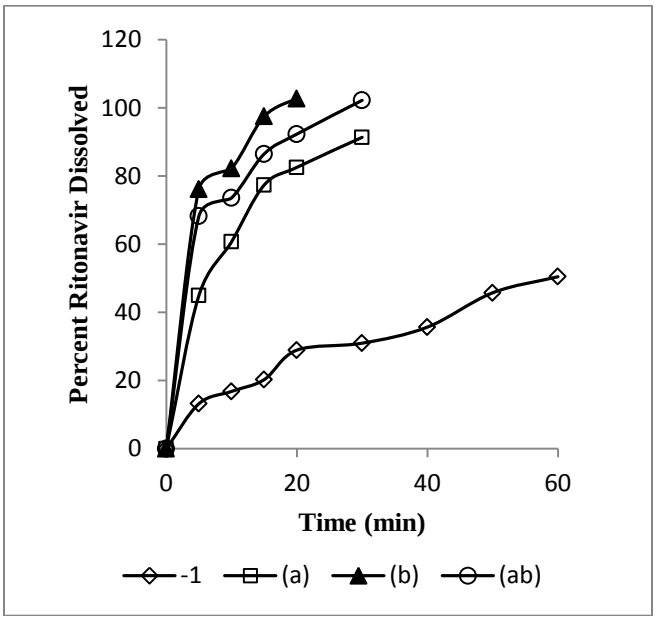

Fig 1: Dissolution Profiles of Ritonavir- βCD- Solutol HS15 Inclusion Complexes Prepared as per 2^2 Factorial Design

The dissolution of ritonavir followed first order kinetics with r (correlation coefficient) above 0.936. Dissolution efficiency (DE_{30}) values were calculated as suggested by Khan[8]. The dissolution parameters are given in Table-3. The dissolution of ritonavir was rapid and higher in the case of ritonavir- βCD - Solutol HS15 complex systems prepared when compared to ritonavir pure drug as such.

ISSN: 0975-8585

Table 3: Dissolution Parameters of Ritonavir – β CD- Solutol HS15 Inclusion Complexes

Formulation Code as per 2^2 Factorial Design	PD_{10} (%)		Dissolution Efficiency DE_{30} (%)		Dissolution Rate $K_1 \times 10^2$ (min^{-1})	
	$\bar{X}$ ± s.d	Increase in PD_{10} (No. of olds)	$\bar{X}$ ± s.d	Increase in DE_{30} (No. of folds)	$\bar{X}$ ± s.d	Increase in K_1 (no. of folds)
1	16.83±3.81	--	20.79±1.42	-	1.070±0.03	-
A	60.71±0.54	3.61	66.38±0.81	3.19	8.360±1.68	7.81
B	82.25±0.98	4.89	85.26±1.26	4.1	17.02±0.40	15.90
Ab	73.60±2.50	4.37	78.18±2.03	3.76	12.62±2.98	11.79

Table 4: ANOVA of Dissolution Rate (K_1) Data

Source of Variation	D F	S.S	MSS (SS/DF)	F – Ratio
Total	11	439.08	39.91	
Treatment	3	415.27	138.42	46.52
Error	8	23.80	2.97	
Factor A	1	6.27	6.27	2.10
Factor B	1	306.53	306.53	103.01
Factor AB	1	102.48	102.48	34.44

$F_{0.05}$ (3, 8) =4.07; $F_{0.05}$ (1, 8) = 5.32; $F_{0.01}$(3, 8) = 7.59; $F_{0.01}$(1, 8) = 11.3

Table 5: ANOVA of Dissolution Efficiency (DE_{30}) Data

Source of Variation	D F	S.S	MSS (SS/DF)	F – Ratio
Total	11	7572.72	688.43	
Treatment	3	7555.90	2518.63	1197.64
Error	8	16.82	2.10	
Factor A	1	1112.79	1112.79	529.14
Factor B	1	4362.23	4362.23	2074.29
Factor AB	1	2080.89	2080.89	989.48

$F_{0.05}$ (3, 8) =4.07; $F_{0.05}$ (1, 8) = 5.32; $F_{0.01}$(3, 8) = 7.59; $F_{0.01}$(1, 8) = 11.3

The dissolution rate (K_1) and dissolution efficiency (DE_{30}) values were subjected to ANOVA to find out the significance of the main and combined effects of βCD and Solutol HS 15 on the dissolution rate and dissolution efficiency of ritonavir. The results of ANOVA of dissolution rate (K_1) values (Table 4) indicated that the individual main effect of Solutol HS15 (Factor B) and combined (interaction) effect of βCD and Solutol HS15 (Factor AB) in enhancing the dissolution rate (K_1) were highly significant ($P < 0.01$). The individual effect of βCD (Factor A) in enhancing the dissolution rate (K_1) was not significant ($P > 0.05$). Whereas the individual and combined effects of βCD and Solutol HS15 in enhancing the dissolution efficiency (DE_{30}) were highly significant ($P < 0.01$).

βCD alone gave a 7.81 fold increase in the dissolution rate of (K_1) of ritonavir and in combination with Solutol HS15 it gave a 11.79 fold increase in the dissolution rate (K_1) of ritonavir. Solutol HS15 alone gave higher enhancement in the dissolution rate (15.90 fold) and dissolution efficiency (4.1 fold) of ritonavir. Solutol HS15 alone or a combination of βCD and Solutol HS15 were found to be more suitable to enhance the dissolution rate and dissolution efficiency of ritonavir.

CONCLUSIONS

- The individual and combined effects of βCD and Solutol HS15 in enhancing the solubility of ritonavir were highly significant ($P < 0.01$).
- βCD and Solutol HS15 alone gave a 4.66 and 13.57 fold increase in the solubility of ritonavir respectively. Combination of βCD with Solutol HS15 resulted in a much higher enhancement in the solubility of ritonavir, 15.92 fold.
- The individual main effect of Solutol HS15 (Factor B) and combined (interaction) effect of βCD and Solutol HS15 (Factor AB) in enhancing the dissolution rate (K_1) were highly significant ($P < 0.01$), whereas the individual effect of βCD (Factor A) was not significant ($P > 0.05$).
- βCD alone gave a 7.81 fold increase in the dissolution rate of (K_1) of ritonavir and in combination with Solutol HS15 it gave a 11.79 fold increase in the dissolution rate (K_1) of ritonavir.
- Solutol HS 15 alone gave higher enhancement in the dissolution rate (15.90 fold) and dissolution efficiency (4.1 fold) of ritonavir.
- Hence Solutol HS 15 alone or a combination of βCD and Solutol HS 15 is recommended to enhance the solubility, dissolution rate and dissolution efficiency of ritonavir, a poorly soluble BCS class II drug.

REFERENCES

[1] Chowdary KPR and Madhavi BLR. Indian Drugs 2005; 42(9): 557-562.
[2] Fromming KH and Szejtli J. Cyclodextrins in Pharmacy, Kluwer Academic Publications, Dordrecghi, p. 20, 1994.
[3] Duchene D and Woussidjewe D in ed. S Dumitriu. Polysaccharides in Medical Applications, Marcel Dekker, New York, p. 575, 1996.
[4] Thompson DO. Crit Rev Ther Drug Carrier Syst 1997; 14 (1): 1-104.
[5] Hedges AR. Chem Rev 1998; 98: 2035.
[6] Sherry Ku and Ranga. Velagaleti Pharm Technol 2010; 2: 108-110.
[7] Rajebahadur M, Zia H, Nues A and Lee C. Drug Delivery 2006; 13(3): 201-6.
[8] Khan KA. J Pharm Pharmacol 1975; 27: 48-49.