INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ENHANCEMENT OF SOLUBILITY, DISSOLUTION RATE AND BIOAVAILABILITY OF RITONAVIR BY CYCLODEXTRINS AND SOLUTOL HS15 - A FACTORIAL STUDY

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ABSTRACT

Ritonavir widely prescribed anti-retroviral drug belongs to class II BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. Andit requires enhancement in solubility and dissolution rate for increasing its oral bioavailability.The objective of the present investigation is to enhance the solubility, dissolution rate and bioavailability of ritonavir by the use ofcyclodextrins (βCD and HPβCD) and surfactant,Solutol HS15. The individual main effects and combined (interaction) effects of cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 on the solubility and dissolution rate of Ritonavirwere evaluated in a series of 2^2 factorial experiments. The solubility of Ritonavir in the fluids containing βCD, HPβCD and Solutol HS15 as per 2^2 factorial design was determined. Ritonavir-CD-surfactant complex systems were prepared employing selected combinations of CDs and surfactant in each case as per a 2^2 factorial design by kneading method.

The results of the present investigation clearly indicated that the individual main effects as well as combined effects of CDs (βCD and HPβCD) and surfactant Solutol HS15 in enhancing the solubility and dissolution rate (K_1) of ritonavir are highly significant ($P < 0.01$). Combination of Solutol HS15 with CDs (βCD and HPβCD) resulted in a much higher enhancement in the solubility and dissolution rate (K_1) of ritonavir than is possible with CDs and Solutol HS15 alone. βCD-Solutol HS15 combination gave 28.97 fold increase in the solubility and 8.66 fold increase in the dissolution rate(K_1) of ritonavir.. Hence a combination of cyclodextrins(βCD and HPβCD) and Solutol HS15 is recommended for enhancing the solubility, dissolution rate and bioavailability of ritonavir, a BCS Class II drug.

Keywords: Ritonavir, Solubility, Dissolution rate, Bioavailability, Cyclodextrins.

INTRODUCTION

The most important property of a drug delivery system is its ability to deliver the active pharmaceutical ingredient (API) to the site of action in the body in an amount sufficient to produce the desired therapeutic response. This property of the drug delivery system is referred to as bioavailability. Bioavailability is more precisely defined as the rate and extent of absorption (availability) of drug to the systemic circulation. About 95 % of all new potential therapeutics (APIs) exhibit low and variable oral bioavailability due to their poor aqueous solubility at physiological pHs and consequent low dissolution rate. These drugs are classified as class II drugs under Bio-Pharmaceutical System (BCS) and pose challenging problems in their pharmaceutical product development process.

Several modern organic drugs belong to class II category under BCS and exhibit low and variable dissolution rates. These drugs need

enhancement in dissolution rate and bioavailability to derive their maximum therapeutic efficacy. Several conventional methods such as micronization, chemical modification, use of surfactants and solubilizers, solid dispersion and a few new emerging technologies such as cyclodextrincomplexation, mucoadhesive microspheres, nanoparticles, nanosuspensions, micro emulsion and self-emulsifying systems are available to enhance the bioavailability of BCS Class II drugs.

Ritonavir a widely prescribed anti-retroviral drug belongs to class II BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility[1]. It is practically in soluble 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. 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[2,3]. As a consequence of inclusion process many physico-chemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably affected.[4,5] Cyclodextrins have been receiving increasing application in pharmaceutical formulation in recent years due to their approval by various regulatory agencies[6,7]. Surfactants also increase the solubility of lipophilic water-insoluble drugs by micellarsolubilization. Though cyclodextrincomplexation and use of surfactants 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.

The objective of the present investigation is to enhance the solubility, dissolution rate and bioavailability of ritonavir by the use ofcyclodextrins (βCD and HPβCD) and surfactant,Solutol HS15. The individual main effects and combined (interaction) effects of cyclodextrins (βCD and HPβCD) and surfactant Solutol HS15 on the solubility and dissolution rate of ritonavir were evaluated in a series of 2^2 factorial experiments.

EXPERIMENTAL

Materials

Ritonavir was a gift sample from M/s Amoli Organics Pvt., Ltd., Mumbai,β-cyclodextrin and hydroxypropyl β-cyclodextrin were gift samples from Signet Chemical Corporation Pvt., Ltd., Mumbai..Solutol HS15 was a gift sample from Dr. Reddy's Laboratories Ltd, Hyderabad.Polyvinyl pyrrolidone (PVP K-30) and Crosscarmellose sodium were gift samples from M/s NatcoPharma Ltd., Hyderabad. Talc, I.P. Magnesium stearate, I.P. and Lactose, I.P.were procured from commercial sources. All other materials used were of pharmacopoeial grade.

Methods

Determination of solubility

The solubility of ritonavir in the following four selected fluids as per 2^2 factorial study was determined to evaluate the individual and combined effects of the cyclodextrins and the surfactant on the solubility of ritonavir. The two levels of CD (factor a) are 0 and 5 mM. The two levels of surfactant (factor b) are 0 and 2%.

The selected fluids as per 2^2 factorial study are as follows

For Ritonavir- βCD-Solutol HS15 system

Statistical code as per 2^2 – Factorial Design	Description
(1)	Purified water
(a)	Water containing βCD (5 mM)
(b)	Water containing Solutol HS15 (2 %)
(ab)	Water containing βCD (5 mM) and Solutol HS15 (2 %)

For Ritonavir- HPβCD-Solutol HS15 system

Statistical code as per 2^2 – Factorial Design	Description
(1)	Purified water
(a)	Water containing HPβCD (5 mM)
(b)	Water containing Solutol HS15 (2 %)
(ab)	Water containing HPβCD (5 mM) and Solutol HS15 (2 %)

Procedure
Excess drug was added to 15 ml of the selected fluid taken in a 25ml stoppered conical flask and the mixtures were shaken for 72 h at room temperature (28^0C) on a rotary flask shaker. After 72 hrs of shaking to achieve equilibrium, 2 ml of aliquots were withdrawn and filtered immediately using 0.45µ disc filter. The filtered samples were diluted suitably and assayed at 210nm. In each case the solubility determinations were replicate 4 times (n=4).

Preparation of Drug -CD- Surfactant systems
To evaluate the individual and combined effects of cyclodextrinsand surfactant on the dissolution rate of ritonavir drug-CD-surfactant systems were prepared employing the following selected combinations of CD and surfactant in each case as per a 2^2 factorial design. The two levels of CD (factor a) are 0 and 1:2 ratio of drug: CD respectively. The two levels of surfactant (factor b) are 0 and 2%. The following are the selected treatments as per 2^2 factorial design in each case to evaluate the individual and combined effects.
The selected treatments (products) as per 2^2 – factorial study in each case are as follows.

For Ritonavir- βCD-Solutol HS15 system

Statistical code as per 2^2 – Factorial Design	Description
(1)	Ritonavir pure drug
(a)	Ritonavir-βCD (1:2) binary system
(b)	Ritonavir-Solutol HS15 (2 %) binary system
(ab)	Ritonavir-βCD-Solutol HS15 (1:2:0.02) ternary system

For Ritonavir- HPβCD-Solutol HS15 system

Statistical code as per 2^2 – Factorial Design	Description
(1)	Ritonavir pure drug
(a)	Ritonavir-HPβCD (1:2) binary system
(b)	Ritonavir-Solutol HS15 (2 %) binary system
(ab)	Ritonavir-HPβCD-Solutol HS15 (1:2:0.02) ternary system

The above mentioned binary and ternary systems were prepared by kneading method employing βCD, HPβCD and Solutol HS15.

Preparation method
Required quantities of drug, βCD and surfactant were taken in a clean and dry mortar. Kneading fluid consisting of water: alcohol (1:1) was added and mixed to get a thick slurry. The slurry was thoroughly mixed and kneaded for 45 min .Additional quantities of kneading fluid was added to maintain the mixture as thick slurry during the kneading process. After kneading for 45 min the mixture was transferred to a petridish and dried in an oven at 60^0C. The dried powder was passed through mesh No.100.

Estimation of drug content in drug-CD-surfactant complexes prepared
Drug-CD-surfactant complex powder equivalent to 50 mg of the medicament was taken into a boiling test tube and extracted with 4 x 10 ml quantities of methanol. The methanolic extracts were collected into 50 ml volumetric flask and the volume was made upto 50 ml with methanol. The solution was subsequently diluted with 0.1N HCLand assayed for the drug content by the UV spectrophotometric method.

Dissolution Rate study on Drug-CD-Surfactant Systems
The dissolution rate of medicament from the drug-CD-surfactant systems prepared was studied in 0.1N HCL (900 ml) using Disso 2000 (Labindia) 8-station dissolution test apparatus with a paddle stirrer at 50 rpm. A temperature of 37°C ± 1°C was maintained throughout the study. Complex system equivalent to 50 mg of drug was used in each test. Samples of dissolution media (5 ml) were withdrawn through a filter (0.45µ) at different intervals of time, suitably 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 four times each (n=4).

RESULTS AND DISCUSSION

Ritonavir, a widely prescribed anti retroviraldrug is poorly soluble in water and aqueous fluids and exhibit low and variable oral bioavailability. It require enhancement in solubility and dissolution rate for increasing its oral bioavailability. In the present study two cyclodextrins (βCD and HPβCD) and a surfactant (SOLUTOL HS15) were tried to enhance the solubility, dissolution rate and bioavailability of ritonavir. The individual main effects and combined (interaction) effects of cyclodextrins and the surfactant on the solubility and dissolution rate of ritonavir were evaluated in a series of 2^2 factorial experiments. The results of solubility studies with βCD and Solutol HS15 are given in Table (1).

The solubility of ritonavir was markedly enhanced by βCD and Solutol HS15. A 1.57 and 21.72 fold increase in the solubility of ritonavir was observed respectively with βCD (5mM) and Solutol HS15 (2%) when used alone. A combination of βCD (5mM) and Solutol HS15 (2%) gave a 28.97 fold increase in the solubility of ritonavir. The solubility data were subjected to Analysis of Variance (ANOVA) to find out the significance of individual main and combined (interaction) effects of βCD and SolutolHS15 on the solubility of ritonavir. ANOVA indicated that the individual main effects of βCD and Solutol HS15 as well as the combined effects are highly significant (P<0.01). A combination of βCD and Solutol HS15 has resulted in a much higher enhancement on the solubility of ritonavir than is possible with them individually. This may be due to better inclusion of drug molecules in the presence of Solutol HS15.The results of solubility studies with HPβCD and Solutol HS15 are given in Table(2).

The solubility of ritonavir was markedly enhanced by HPβCD and Solutol HS15. A 1.47 and 21.72 fold increase in the solubility of ritonavir was observed respectively with HPβCD (5mM) and Solutol HS15 (2%). A combination of HPβCD (5mM) and Solutol HS15 (2%) gave a 22.31 fold increase in the solubility of ritonavir. ANOVA indicated that the individual main effects of Solutol HS15 are highly significant (p<0.01) whereas individual main effects of HPβCD and combined effects of HPβCD (5mM) and Solutol HS15 (2%) are not significant (p>0.01)

The order of increasing enhancement observed with various CDs and Surfactant was Solutol HS15 > βCD >HPβCD. Among all the combinations, βCD-Solutol HS15 exhibited greater enhancement in the aqueous solubility (28.97 fold) of ritonavir.

To evaluate the individual main and combined effects of cyclodextrins (βCD and HPβCD) and surfactant, Solutol HS15on the dissolution rate of ritonavir, solid inclusion complexes of Drug-CD-Surfactant were prepared in each case as per 2^2 factorial design. All the solid inclusion complexes prepared were found to be fine and free flowing powders. Low C.V values (< 1.5%) in the percent drug content indicated uniformity of drug content in each batch of solid inclusion complexes prepared.

The dissolution rate of ritonavirfrom various Drug-CD-Surfactant complexes prepared was studied in 0.1N HCL.Dissolution data were analyzed as per zero and first order kinetics. The correlation coefficient (r^2) values in the analysis of dissolution data indicated that the dissolution of ritonavir from all the tablets formulated followed first order kinetics. The correlation coefficient (r^2) values were in the range 0.8927-0.9978 with all the ritonavir tablets prepared.

The dissolution rate constants were calculated in each case separately for 0-5 min. and 5-30 min. and the average of the two was calculated and reported as dissolution rate (K_1). The first order dissolution rates (K_1) and Dissolution efficiency (DE_{15}) values, calculated as per Khan[8]. The dissolution rates (K_1) and Dissolution efficiency (DE_{15}) values were several times higher in the case of CD-Surfactant complexes when compared to ritonavir pure drug.The Increase in DE_{15} and K_1 values of ritonavir by βCD and βCD – Solutol HS15 are given in Table(3).

Among the individual effects, CDs (βCD and HPβCD) gave higher enhancement in the K_1 and DE_{15} of ritonavir than the surfactant Solutol HS15. The order of increasing enhancement in dissolution rate (K_1) observed with various CDs and surfactants was βCD > HPβCD > Solutol HS15. The order of increasing enhancement in dissolution efficiency (DE_{15}) observedwith various CDs and surfactants wasHPβCD > βCD > Solutol HS15.

The dissolution rate (K_1) values of various Drug-CD-Surfactant complex systems were subjected to Analysis of Variance (ANOVA) to evaluate the significance of the individual main and combined effects of CDs and surfactants in enhancing the dissolution rate (K_1) of ritonavir. The results of ANOVA indicated that all individual and combined effects were highly significant ($P < 0.01$). Among the combined effects, βCD-Solutol HS15 gave highest enhancement in K_1 (8.66 fold). (K_1) of ritonavir.

CONCLUSION

The results of the present investigation clearly indicated that the individual main effects as well as combined effects of CDs (βCD and HPβCD) and surfactant Solutol HS15 in enhancing the solubility and dissolution rate (K_1) of ritonavir are highly significant ($P < 0.01$). Combination of Solutol HS15 with CDs (βCD and HPβCD) resulted in a much higher enhancement in the solubility and dissolution rate (K_1) of ritonavir than is possible with CDs and Solutol HS15 alone. βCD-Solutol HS15 combination gave 28.97 fold increase in the solubility and 8.66 fold increase in the dissolution rate(K_1) of ritonavir.

Hence a combination of cyclodextrins(βCD and HPβCD) and Solutol HS15 is recommended for enhancing the solubility, dissolution rate and bioavailability of Ritonavir, a BCS Class II drug.

Table 1: Solubility of Ritonavir in Various Fluids (N=4)as Per 2^2 Factorial Study (Ritonavir-βCD-Solutol HS15)

Fluid	Solubility (mg/100 ml) $\bar{x}$ ± sd	Increase in solubility (no. of folds)
Purified water	5.42 ± 0.184	-
Water containing βCD (5mM)	8.53 ± 0.311	1.57
Water containing Solutol HS15 (2%)	117.74 ± 5.54	21.72
Water containing βCD (5mM) and Solutol HS15 (2%)	157.06 ± 10.48	28.97

Table 2: Solubility of Ritonavir in Various Fluids (N=4) as Per 2^2 Factorial Study (Ritonavir-HPβCD-Solutol HS15)

Fluid	Solubility (mg/100 ml) $\bar{x}$ ± sd	Increase in solubility (no. of folds)
Purified water	5.42 ± 0.184	-
Water containing HPβCD(5mM)	6.32 ± 0.29	1.47
Water containing Solutol HS15 (2%)	117.74 ± 5.91	21.72
Water containing HPβCD (5mM) and Solutol HS15 (2%)	120.96 ± 2.39	22.31

Table 3: Increase in DE_{15} and K_1 values of Ritonavir by βCD and βCD –Solutol HS15

CD complex	DE_{15} (%)		$K_1 \times 10^2$ (min^{-1})	
	$\bar{x}$	Increase (no. of folds)	$\bar{x}$	Increase (no. of folds)
Ritonavir	10.11	-	5.27	-
βCD	63.87	6.31	45.66	8.66
HPβCD	69.09	6.83	44.88	8.51
Solutol HS15	52.17	5.16	40.85	7.75
βCD-Solutol HS15	73.89	7.30	45.66	8.66
HPβCD-Solutol HS15	63.67	6.29	44.23	8.39

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