FORMULATION OF RITONAVIR TABLETS: OPTIMIZATION BY $2^2$ FACTORIAL DESIGN

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ABSTRACT

Ritonavir, a widely prescribed antiretroviral drug belongs to class II under BCS classification and exhibit low and variable oral bioavailability due to its poor aqueous solubility. It needs enhancement in the dissolution rate in its formulation development. Complexation with β-cyclodextrin (βCD) and use of surfactant (Soluplus) are tried for enhancing the dissolution rate of ritonavir in its formulation development. The objective of the present study is optimization of ritonavir tablet formulation employing βCD and Soluplus by $2^2$ factorial design. Formulation of ritonavir tablets with NLT 85% dissolution in 15 min employing βCD and Soluplus was optimized by $2^2$ factorial design. Four ritonavir (100 mg) tablet formulations were prepared using selected combinations of the two factors as per $2^2$ factorial design. Ritonavir tablets were prepared by direct compression method and were evaluated for drug content, hardness, friability, disintegration time and dissolution rate characteristics. All the ritonavir tablets prepared fulfilled the official (IP 2010) requirements with regard to drug content, hardness, friability and disintegration time specified for uncoated tablets. Much variations were observed in the disintegration and dissolution characteristics of the ritonavir tablets prepared due to formulation variables. The disintegration times were in the range 30 sec to 6 min 15 sec with various tablets. Ritonavir tablets (Rb) which are prepared employing βCD in 1:0.5 ratio of drug: βCD and Soluplus at 2% of drug content gave very rapid dissolution of ritonavir than others. These tablets (Rb) gave 99.50% dissolution in 15 min. The increasing order of dissolution rate ($K_1$) observed with various formulations was Rb>Rab>Ra>R1. For optimization, percent drug dissolved in 15 min was
taken as response ($Y$) and level of βCD as ($X_1$) and level of Soluplus as ($X_2$). The polynomial equation describing the relationship between the response, $Y$ and the variables, $X_1$ and $X_2$ based on the observed data was found to be $Y = 77.48 + 4.42 (X_1) + 16.7 (X_2) - 9.67 (X_1 X_2)$. Based on the above polynomial equation, the optimized ritonavir tablet formulation with NLT 85% dissolution in 15 min could be formulated employing βCD at 1:2.75 ratio of drug: βCD and Soluplus at 1.50 % of drug content. The optimized ritonavir (100 mg) tablet formulation prepared employing βCD (275 mg / tablet) and Soluplus (1.5 mg / tablet) gave 85.95 % dissolution in 15 min fulfilling the target dissolution set. Thus optimization by $2^2$ factorial design could be successfully used for the development of ritonavir tablets with NLT 85 % dissolution in 15 min.

**KEYWORDS:** Ritonavir tablets, Optimization, β-cyclodextrin, Soluplus, Factorial Design.

**INTRODUCTION**

Majority of modern organic drugs exhibit low and variable oral bioavailability due to their poor aqueous solubility at physiological pH and consequent low dissolution rate. These drugs are classified as class II drugs under BCS with low solubility and high permeability characters. These BCS class II drugs pose challenging problems in their pharmaceutical product development process. Ritonavir, a widely prescribed antiretroviral drug belongs to class II under BCS classification and exhibit low and variable oral bioavailability due to its poor aqueous solubility. Because of poor aqueous solubility and dissolution rate it poses challenging problems in its tablet formulation development. It needs enhancement in the dissolution rate in its formulation development.

Several techniques,\cite{1} such as micronisation, cyclodextrin-complexation, use of surfactants, solubilizers and super disintegrants, solid dispersion in water soluble and water dispersible carriers, microemulsions and self emulsifying micro and nano disperse systems are reported to enhance the solubility, dissolution rate and bioavailability of poorly soluble BCS class II drugs. Among the various approaches complexation with cyclodextrins and use of surfactants have 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.\cite{2,3} Cyclodextrins have been receiving increasing application in
pharmaceutical formulation in recent years due to their approval by various regulatory agencies\cite{4,5} Soluplus is polyvinyl caprolactam – polyvinyl acetate – polyethylene glycol graft co-polymer. Soluplus was reported\cite{6,7} to increase the solubility, dissolution rate and bioavailability of drugs such as itraconazole, fenofibrate and valsartan.

Complexation with β-cyclodextrin (βCD) and use of Soluplus (a non-ionic surfactant) are tried for enhancing the dissolution rate of ritonavir in its formulation development. The objective of the present study is optimization of ritonavir tablet formulation employing βCD and Soluplus by $2^2$ factorial design. Formulation of ritonavir tablets with NLT 85% dissolution in 15 min employing βCD and Soluplus was optimized by $2^2$ factorial design.

Optimization\cite{8} of pharmaceutical formulations involves choosing and combining ingredients that will result in a formulation whose attributes confirm with certain prerequisite requirements. The choice of the nature and qualities of additives (excipients) to be used in a new formulation shall be on a rational basis. The optimization procedure is facilitated by applying factorial designs and by the fitting of an empirical polynomial equation to the experimental results. The predicted optimal formulation has to be prepared and evaluated to confirm its quality. The objective of the present study is optimization of ritonavir tablet formulation employing βCD and Soluplus by $2^2$ factorial design.

**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. 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 0.1N 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.
Formulation of Ritonavir Tablets

For optimization of ritonavir tablets as per $2^2$ factorial design, the βCD and Soluplus are considered as the two factors. The two levels of the factor A (βCD) are 1:0.5 and 1:5 ratio of drug: βCD and the two levels of the factor B (Soluplus) are 0.2% and 2% of drug content. Four ritonavir tablet formulations employing selected combinations of the two factors i.e. βCD and Soluplus as per $2^2$ factorial design were formulated and prepared by direct compression method.

Preparation of Ritonavir Tablets

Ritonavir (100 mg) tablets were prepared by direct compression method as per the formula given in Table 1.

Ritonavir - βCD - Soluplus inclusion complexes involved in the formulation of ritonavir tablets were initially prepared by kneading method. Ritonavir, βCD and Soluplus 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. The solid inclusion complexes prepared and all other ingredients as per the formula in each case were blended in a closed polyethylene bag and were compressed into tablets using an 8- station RIMEK tablet punching machine employing 9 mm or 12 mm round and flat punches to a hardness of 4- 6 kg/cm$^2$. In each case 100 tablets were compressed.

Evaluation of Tablets

All the ritonavir tablets prepared were evaluated for drug content, hardness, friability, disintegration time and dissolution rate as follows.

Hardness

The hardness of prepared tablets was determined by using Monsanto hardness tester and measured in terms of kg/cm$^2$.

Friability

The friability of the tablets was measured in a Roche friabilator using the formula Friability ($\%$) = [(Initial weight- Final weight) / (Initial weight)] x 100.
Drug Content
Weighed tablets (5) were powdered using a glass mortar and pestle. An accurately weighed quantity of powder equivalent to 20 mg of ritonavir was taken into 100 ml volumetric flask, dissolved in 0.1 N HCl and the solution was filtered through Whatman filter paper no.41. The filtrate was collected and suitably diluted 0.1 N HCl and assayed for ritonavir at 240 nm.

Disintegration time
Disintegration time of the tablets was determined using single unit disintegration test apparatus (Make: Paramount) employing water as test fluid.

Dissolution Rate Study
Dissolution rate of ritonavir tablets prepared was studied in 0.1 N HCl (900 ml) employing eight station dissolution rate test apparatus (LABINDIA, DS 8000) using paddle stirrer at 50 rpm and at a temperature of 37°C ± 1°C. One tablet was used in each test. Samples of dissolution fluid (5 ml) were withdrawn through a filter at different time intervals and assayed for ritonavir at 240 nm. The sample of dissolution fluid withdrawn at each time was replaced with fresh drug free dissolution fluid and a suitable correction was made for the amount of drug present in the samples withdrawn in calculating percent dissolved at various times. Each dissolution experiment was run in triplicate (n=3).

Analysis of Data
The dissolution data were analyzed as per zero order and first order kinetic models. Dissolution efficiency (DE 30) values were estimated as suggested by Khan.[9]

RESULTS AND DISCUSSION
The objective of the present study is to optimize the ritonavir tablet formulation employing βCD and Soluplus by 2² factorial design to achieve NLT 85% dissolution in 15 min. According to BCS guidance of USFDA and WHO,[10-11] a drug product is considered to be very rapidly dissolving when not less than 85% of the labelled amount of the drug dissolved in 15 min at the three physiological pH’s of 1.2, 4.5 and 6.8. Hence target dissolution to be achieved is fixed at NLT 85% dissolution in 15 min in the formulation development of ritonavir tablets.

For optimization of ritonavir tablets as per 2² factorial design the βCD and Soluplus are considered as the two factors. The two levels of the factor A (βCD) are 1: 0.5 and 1:5 ratio of
drug: βCD and the two levels of the factor B (Soluplus) are 0.2% and 2.0 % of drug content. Four ritonavir tablet formulations employing selected combinations of the two factors i.e. βCD and Soluplus as per $2^2$ factorial design were formulated and prepared by direct compression method as per the formulae given in Table 1. The ritonavir tablets prepared were evaluated for various physical parameters and dissolution rate characteristics.

The physical parameters of the ritonavir tablets prepared are given in Table 2. The hardness of the tablets was in the range 4.0-5.5 kg/cm². Weight loss in the friability test was less than 0.95 % in all the cases. Ritonavir content of the tablets prepared was within 100±3 %. Much variations were observed in the disintegration and dissolution characteristics of the ritonavir tablets prepared. The disintegration times were in the range 30 sec to 6 min 15 sec. All the ritonavir tablets prepared fulfilled the official (IP 2010) requirements with regard to drug content, hardness, friability and disintegration time specified for uncoated tablets.

Dissolution rate of ritonavir tablets prepared was studied in 0.1 N HCl. The dissolution profiles of the tablets are shown in Fig.1 and the dissolution parameters are given in Table 3. Dissolution of ritonavir from all the tablets prepared followed first order kinetics with coefficient of determination ($R^2$) values above 0.925. The first order dissolution rate constant ($K_1$) values were estimated from the slope of the first order linear plots. Variations were observed in the dissolution rate ($K_1$) and $DE_{30}$ values of the tablets prepared due to formulation variables.

Ritonavir tablets (Rb) which are prepared employing βCD in 1:0.5 ratio of drug: βCD and Soluplus at 2 % of drug content gave very rapid dissolution of ritonavir than others. These tablets (Rb) gave 99.50 % dissolution in 15 min. The increasing order of dissolution rate ($K_1$) observed with various formulations was Rb>Rab> Ra > R1.

For optimization, percent drug dissolved in 15 min was taken as response (Y) and level of βCD as ($X_1$) and level of Soluplus as ($X_2$). The polynomial equation describing the relationship between the response, Y and the variables, $X_1$ and $X_2$ based on the observed data was found to be $Y = 77.48 + 4.42 \ (X_1) + 16.7 \ (X_2) − 9.67 \ (X_1 \ X_2)$. Based on the above polynomial equation, the optimized ritonavir tablet formulation with NLT 85% dissolution in 15 min could be formulated employing βCD at 1:2.75 ratio of drug: βCD and Soluplus at 1.50 % of drug content. To verify, ritonavir (100 mg) tablets (Ropt ) were formulated employing the optimized levels of βCD (275 mg/tablet) and Soluplus (1.5 mg/ tablet). The
formula of the optimized ritonavir tablets is given in Table 1. The optimized ritonavir tablet formulation was prepared by direct compression method and the tablets were evaluated. The physical parameters of the optimized formulation are given in Table 2 and dissolution parameters are given in Table 3. The hardness of the optimized ritonavir tablets was 4.5-5.0 kg/sq.cm. Friability (percent weight loss) was less than 0.85 %. The optimized tablets disintegrated in 20 sec. The optimized ritonavir tablet formulation, (Ropt ) gave 85.95 % dissolution in 15 min fulfilling the target dissolution set. Hence optimization by 2² factorial design could be successfully used for the development of ritonavir tablets with NLT 85 % dissolution in 15 min.

**Table 1: Formulæ of Ritonavir Tablets Prepared as per 2² Factorial Design Employing βCD and Soluplus and Optimized Formulation.**

<table>
<thead>
<tr>
<th>Ingredient (mg/tab)</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Ropt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritonavir</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>βCD</td>
<td>50</td>
<td>500</td>
<td>50</td>
<td>500</td>
<td>275</td>
</tr>
<tr>
<td>Soluplus</td>
<td>0.2</td>
<td>0.2</td>
<td>2.0</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Crosspovidone</td>
<td>9.2</td>
<td>28</td>
<td>9.2</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>Talc</td>
<td>2.3</td>
<td>7.0</td>
<td>2.3</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2.3</td>
<td>7.0</td>
<td>2.3</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Aerosil</td>
<td>1.15</td>
<td>3.5</td>
<td>1.15</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Avicel PH 102</td>
<td>64.85</td>
<td>54.3</td>
<td>63.05</td>
<td>52.50</td>
<td>91.0</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>230</td>
<td>700</td>
<td>230</td>
<td>700</td>
<td>500</td>
</tr>
</tbody>
</table>

**Table 2: Physical Parameters of Ritonavir Tablets Prepared as per 2² Factorial Design Employing βCD and Soluplus and Optimized Formulation.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness (Kg/cm²)</th>
<th>Friability (% Wt loss)</th>
<th>Disintegration Time (min-sec)</th>
<th>Drug Content (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>5.0</td>
<td>0.85</td>
<td>0-30</td>
<td>98.6</td>
</tr>
<tr>
<td>R₂</td>
<td>5.5</td>
<td>0.65</td>
<td>6-15</td>
<td>99.2</td>
</tr>
<tr>
<td>R₃</td>
<td>4.0</td>
<td>0.75</td>
<td>0-55</td>
<td>98.5</td>
</tr>
<tr>
<td>R₄</td>
<td>4.5</td>
<td>0.85</td>
<td>1-45</td>
<td>99.5</td>
</tr>
<tr>
<td>Ropt</td>
<td>4.5</td>
<td>0.85</td>
<td>0-20</td>
<td>98.7</td>
</tr>
</tbody>
</table>

**Table 3: Dissolution Parameters of Ritonavir Tablets Prepared as per 2² Factorial Design Employing βCD and Soluplus and Optimized Formulation.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>PD₁₅ (%)</th>
<th>DE₃₀ (%)</th>
<th>K₁ X 10⁻² (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>46.70</td>
<td>40.70</td>
<td>2.74</td>
</tr>
<tr>
<td>R₂</td>
<td>74.90</td>
<td>64.30</td>
<td>3.36</td>
</tr>
<tr>
<td>R₃</td>
<td>99.50</td>
<td>89.40</td>
<td>45.5</td>
</tr>
<tr>
<td>R₄</td>
<td>89.00</td>
<td>79.16</td>
<td>4.67</td>
</tr>
<tr>
<td>Ropt</td>
<td>85.95</td>
<td>78.77</td>
<td>7.18</td>
</tr>
</tbody>
</table>
CONCLUSIONS
1. All the ritonavir tablets prepared fulfilled the official (IP 2010) requirements with regard to drug content, hardness, friability and disintegration time specified for uncoated tablets.
2. Much variations were observed in the disintegration and dissolution characteristics of the ritonavir tablets prepared due to formulation variables.
3. The disintegration times were in the range 30 sec to 6 min 15 sec with various tablets.
4. Ritonavir tablets (Rb) which are prepared employing βCD in 1:0.5 ratio of drug: βCD and Soluplus at 2% of drug content gave very rapid dissolution of ritonavir than others. These tablets (Rb) gave 99.50% dissolution in 15 min.
5. The increasing order of dissolution rate (K1) observed with various formulations was Rb > Rab > Ra > R1.
6. For optimization, percent drug dissolved in 15 min was taken as response (Y) and level of βCD as (X1) and level of Soluplus as (X2). The polynomial equation describing the relationship between the response, Y and the variables, X1 and X2 based on the observed data was found to be
   \[ Y = 77.48 + 4.42 (X_1) + 16.7 (X_2) - 9.67 (X_1 X_2) \].
7. Based on the above polynomial equation, the optimized ritonavir tablet formulation with NLT 85% dissolution in 15 min could be formulated employing βCD at 1:2.75 ratio of drug: βCD and Soluplus at 1.5% of drug content
8. The optimized ritonavir (100 mg) tablet formulation prepared employing βCD (275 mg / tablet) and Soluplus (1.5 mg/ tablet) gave 85.95% dissolution in 15 min fulfilling the target dissolution set.
9. Thus optimization by \(2^2\) factorial design could be successfully used for the development of ritonavir tablets with NLT 85% dissolution in 15 min.
REFERENCES