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# Design and Evaluation of Novel Dosage Form of Rifampicin and Isoniazid with Improved Functionality

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#### Abstract

The aim of present investigation was to develop a novel dosage form of rifampicin and isoniazid to minimize degradation of rifampicin in acidic medium and to modulate the release of rifampicin in the stomach and isoniazid in intestine. Gastroretentive tablets of rifampicin were prepared by direct compression using polyethylene oxide, calcium carbonate and ascorbic acid. The powder blend and tablets of rifampicin were characterized. Isoniazid tablets, prepared by direct compression using dicalcium phosphate, were enteric coated using hydroxypropylmethylcellulose (HP-55S) and characterized. Two tablets each of rifampicin and isoniazid were put into a hard gelatin capsule (size 00) and characterized for in vitro drug release and in vitro drug degradation studies. Rifampicin was released over 4 h from the novel dosage form. Less than 8% of isoniazid was released from novel dosage form before reaching to intestinal pH 7.4. Complete drug release of isoniazid was observed within 90 m at pH 7.4. The degradation of rifampicin to 3-formyl rifampicin SV (3FRSV) in presence of isoniazid was arrested (less than 0.21% degradation of rifampicin at the end of 120 m) from novel dosage form because of the minimization of physical contact between the two drugs and controlled release of rifampicin in acidic medium.

*Key words*-*Rifampicin, Gastroretentive tablets, Isoniazid, Hydroxypropylmethylcellulose phthalate, Degradation, Modified dissolution apparatus* 

### INTRODUCTION

Tuberculosis is a common and deadly infectious disease caused by mycobacterium, mainly Mycobacterium tuberculosis. Isoniazid is a first-line antitubercular drug. It inhibits the synthesis of mycolic acid in the mycobacterium cell wall. It is never used alone to treat active tuberculosis because of quick resistance to body. The chief adverse reactions associated with isoniazid therapy include rashes, hepatitis, sideroblastic anemia and peripheral neuropathy.

Rifampicin, a novel antitubercular drug, inhibits DNAdependent RNA polymerase in bacterial cells by binding its beta-subunit, thus preventing transcription to RNA and subsequent translation to proteins. Rifampicin is lipophilic in nature and thus is ideal candidate to treat the meningitis form of tuberculosis, which requires distribution to the central nervous system and penetration through the blood-brain barrier. The chief adverse reaction associated with rifampicin therapy is hepatitis.

Combination product of isoniazid and rifampicin are widely prescribed for treatment of tuberculosis. However, major problem of such products is degradation of rifampicin in presence of isoniazid in acidic media because of formation of hydrazone.<sup>1-2</sup> Rifampicin is well absorbed from the stomach because of its high solubility between pH 1-2 whereas isoniazid is well absorbed from all three segments of the intestine.<sup>3</sup>

The basic intention of formulation chemist is to develop bioavailable dosage form. Bioavailability not only depends on solubility and permeability of drugs but it also can be manipulated by properly choosing formulation ingredients. The drug should be released at a site of absorption at a rate which is equal to the absorption rate. The present work was undertaken to prevent degradation of rifampicin in presence of isoniazid in

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acidic medium by formulating novel solid dosage form (tablets within a capsule) comprising of gastro-retentive modified release rifampicin tablets and enteric coated isoniazid tablets.

### MATERIALS AND METHODS Materials:

Rifampicin and isoniazid were received as gift from Sunij Pharma Ltd. (India). Polyethylene oxide (PEO WSR N80), hydroxypropylmethylcellulose phthalate (HP-55S) and dicalcium phosphate dihydrate (DCP) were received as gift from Cadila Pharmaceuticals Ltd. (India). Calcium carbonate, chloroform, acetone and isopropyl alcohol were procured from S.D. Fine Laboratory (India). Anhydrous sodium sulphate was procured from Laser Laboratories (India). Ascorbic acid was purchased from Dewang Corporation (India). Empty hard gelatin capsules were gifted by Capsule Corporation of India (India).

#### Methods:

Rifampicin floating matrix tablets were prepared by direct compression method. Rifampicin (75% w/w), polyethylene oxide (15% w/w), ascorbic acid (1.5% w/w), calcium carbonate (8% w/w) and magnesium stearate (0.5% w/w) were geometrically mixed and compressed to tablet of 200 mg average weight and 70 N crushing strength, by direct compression method on single station tablet machine (Cadmach, India). The powder blend of rifampicin matrix tablet was characterized for angle of repose and Carr's index while the tablets were characterized for percentage friability and *in vitro* drug release. The composition of rifampicin tablets was selected on basis of preliminary studies carried out by varying concentration of polyethylene oxide and calcium carbonate from 10-20% w/w and 3-13 % w/w respectively (data not shown).

The core tablets of isoniazid, with average weight of 230 mg and 80 N crushing strengths were prepared by direct compression. Isoniazid (3 g), DCP (1.5 g) and magnesium stearate (0.1 g) were geometrically mixed in motar and pestle. The composition of isoniazid core tablets was selected on basis of preliminary studies carried out by varying ratio of isoniazid and dicalcium phosphate dihydrate from 1:0.2-1:0.5. The tablets of isoniazid were coated with dispersion of HP-55S using conventional coating pan (Manesty - model number

354255). Three batches were formulated with different weight gain (batch A1-3%, A2-6% and A3-9% respectively) to achieve enteric effect. The dissolution criteria were arbitrarily selected as not more than 10% of the drug release should occur in 120 m at pH 1.5. The coated tablets (batches A1-A3) were characterized for enteric test and *in vitro* drug release. The coating dispersion contained HP-55S (3.5% w/v), dibutyl phthalate (10% w/w of polymer), magnesium stearate and titanium dioxide (1% w/w of polymer) and blend of acetone and isopropyl alcohol blend (1:1, quantity sufficient).

Two gastro-retentive tablets of rifampicin and two enteric coated isoniazid tablets (batch A3) were filled in a "00" size hard gelatin capsules to formulate novel dosage form (Fig. 1) and evaluated for *in vitro* drug release and *in vitro* drug degradation.

#### **EVALUATION:**

The angle of repose was measured using the fixed height funnel method.<sup>4-5</sup>Carr's index was found by adding 2 g of sample to 10 ml measuring cylinder. After noting the initial volume, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 second intervals. The tapping was continued until no further change in volume was noted. Friability was carried out in USP friabilator (Electrolab, Model EF2, India). Lag time to float and duration of floating was measured by adding samples into 900 ml of 0.1 N hydrochloric acid maintained at  $37 \pm 0.5^{\circ}$  C. Enteric test was performed using phosphate buffer (pH 6.8) following storage of the tablets in 0.1N HCl (pH 1.5) for 2 h. The results are depicted in Table 1. The in vitro drug release and in vitro degradation study was carried out using modified dissolution apparatus along USP dissolution test apparatus-II (Electrolab, Model TDT 06-T, India). The modified dissolution apparatus contained a modified glass beaker (Fig. 1) filled with 75 ml of hydrochloric acid (pH 1.5). Hydrochloric acid was added from the top at a flow rate of 2 ml/m. The dissolution media in modified dissolution apparatus was maintained at  $37 \pm 0.5^{\circ}$  C and rotated at 75 rpm (Remi magnetic stirrer, India). After 2 h, isoniazid tablets were removed from modified dissolution apparatus and transferred to USP dissolution test apparatus containing 900 ml of phosphate buffer (pH 7.4,  $37 \pm 0.5^{\circ}$  C). The paddles of USP dissolution apparatus were rotated at 100 rpm. For estimation of rifampicin and its degraded product (3FRSV), ten ml of solutions were withdrawn at different time intervals from the sampling beaker of the modified dissolution apparatus and extracted with 10 ml of chloroform. The aqueous medium and chloroform were then separated using a separating funnel. Anhydrous sodium sulphate was added to adsorb droplets of aqueous solution from chloroform. The absorbance of solution in chloroform was measured by dual wavelength spectrophotometric method.<sup>1</sup> The percentage rifampicin degraded was found using equation 1.<sup>6</sup>

% rifampicin degraded = (% 3FRSV formed\*823)/726) - ----(1)

where, 823 and 726 are molecular weight of rifampicin and 3FRSV respectively. Isoniazid was measured spectrophotometrically at 263 nm.<sup>7</sup> The results of *in vitro* drug release and *in vitro* drug degradation study are shown in Fig. 3 and 4.

The method of Bamba *et al.* was adopted to ascertain kinetics of drug release.<sup>8</sup> *In vitro* drug release data of individual rifampicin tablets were analyzed by different kinetic models in order to evaluate the release mechanism of rifampicin from the tablet.<sup>9</sup> A FORTRAN software, developed in-house, was used. The least value of sum of square of residuals (SSR) and Fischer's ratio (F) were used to select the most appropriate kinetic model.

#### **RESULTS AND DISCUSSION**

Maggi et al. reported that rifampicin is converted to rifampicin quinone at higher pH.<sup>10</sup> Ascorbic acid was added in the rifampicin tablets as antioxidant. Reactive oxygen species oxidize ascorbate first to monodehydroascorbate and then to dehydroascorbate. The nature, concentration and viscosity grade of polymer in a matrix can modify the kinetics of drug release.<sup>11</sup> In the present study, polyethylene oxide (PEO WSR N80) with molecular weight of 2,00,000 was used as a matrixing agent in the formulation of rifampicin tablets to gradually release the rifampicin within 4 h in acidic pH 1.5. Polyethylene oxide is among various hydrophilic polymers that, in presence of water, form hydrogel. Polyethylene oxide has been proposed as alternatives to cellulose derivatives in the production of controlled drug delivery system.<sup>12</sup> Further, polyethylene oxide exhibits mucoadhesive properties which may assist in prolonging

the gastric residence time.13 Calcium carbonate was added as a gas forming agent in formulation of rifampicin tablets. Calcium carbonate reacts with hydrochloric acid present in the stomach and generates carbon dioxide which causes floating of tablet. Preliminary studies were carried out to develop matrix tablet of rifampicin that showed floating lag time less than 3 m and duration of floating greater than 5 h. The floating study was carried out in 0.1N HCl. Five batches were formulated by varying concentration of polyethylene oxide and calcium carbonate from 10-20% w/w and 3-13% w/w respectively (data not shown). The powder blend of rifampicin tablets exhibited good flow characteristics with Carr's index and angle of repose of <15 and  $<30^{\circ}$ respectively. The percentage friability of rifampicin tablets was within the limits (<1%). Rifampicin tablets containing 15% w/w polyethylene oxide and 8% w/w calcium carbonate showed desired lag time to float (2.5 min) and duration of floating (5.1 h) and hence selected for further study. In vitro release study was carried out in modified dissolution apparatus (Fig. 2) designed specifically for floating dosage form to mimic biorelevant conditions prevailing in the body. Dissolution and absorption can occur concurrently in the body. Accumulation of the dissolved drug is expected in the dissolution medium when a USP dissolution apparatus is used leading to faulty results. Fig. 3 shows that rifampicin was gradually released from the tablets in 0.1 N HCl (pH 1.5). The time required to release, 90% of drug  $(t_{90}\%)$  was 216 m. The *in vitro* release data of rifampicin tablet were analyzed for establishing kinetics of drug release. Model fitting was done using an in-house program developed by the authors. Zero-order, firstorder, Higuchi, Hixson-Crowell, Korsmeyer-Peppas and Weibull models were tested. The best fit was shown by Weibull model with least sum of square of residuals (SSR = 0.66) and Fischer's ratio (F = 0.33).

Isoniazid core tablets were formulated using DCP as filler. The composition of isoniazid core tablets was selected on basis of preliminary studied carried out by varying ratio of isoniazid and DCP from 1:0.2-0.5 (data not shown). Concentration of magnesium stearate was fixed to 2.2% of total amount of isoniazid and DCP. Out of different batches, isoniazid tablets formulated using 1:0.5 passed friability test (<1%) and hence selected for

further study. The purpose of depositing the HP-55S over isoniazid core tablets was to prevent interaction between rifampicin and isoniazid in stomach (pH 1.5). Dibutyl phthalate was used as a hydrophobic plasticizer in coating dispersion of HP-55S to avoid water permeation during in vitro release studies. Water soluble plasticizers can favour pore formation under the conditions of dissolution. Fig. 3 shows that the batch A1 failed to show desired lag time of 120 m at pH 1.5. About 55% of the drug was released in 2 h. The problem was rectified by applying thicker coat of HP-55S in subsequent batches (A2-A3). Batch A3 provided desired lag time of 120 m at pH 1.5 with little drug release (6%) in 2 h. Time required to release, 90% of the drug from tablets of batch A3 was 174 min. The results depicted in Table 1 reveals that batch A3 meet the requirements for the enteric test in pH 1.5 (0.1 N HCl). The disintegration time was directly correlated with percentage weight gain. Hence, considering the results of in vitro drug release and enteric test, tablets of batch A3 were selected for formulating novel dosage form.

Novel dosage form was formulated by placing two gastro-retentive tablets of rifampicin and two enteric coated isoniazid tablets of batch A3 into hard gelatin capsule (size 00). Fig. 3 reveals that rifampicin was gradually released from novel dosage form. Time required to release 90% of rifampicin from novel dosage form was 224 m. Less than 8% of isoniazid was released in acidic pH from novel dosage form (Fig. 4). Complete drug release was obtained thereafter within 90 m in pH 7.4. Time required to release 90% of isoniazid from novel dosage form was 180 m. The *in vitro* release of rifampicin and isoniazid from individual tablets and novel dosage form was statistically insignificant with  $t_{calculated} < t_{critcal-onetail}$  at 5% level of significance.

Shishoo et al. reported that 12% of rifampicin degraded

to 3FRSV in acidic medium in 45 m, while 21% of rifampicin degraded in 45 minutes when a rifampicin release study was performed in the presence of isoniazid.<sup>2</sup> Singh et al. reported that 17% to 24% of rifampicin degraded in 0.1 N HCl at 37°C in 50 m when rifampicin was released with isoniazid.<sup>1</sup> Fig. 4 reveals that more than 29% rifampicin degraded at the end of 120 m when a capsule containing powder blend of rifampicin and isoniazid was subjected to in vitro drug degradation study. The higher percentage of degradation from the capsule containing powder blend of rifampicin and isoniazid may be attributed to the triggering action of isoniazid as reported by Shishoo *et al*<sup>1</sup> and Singh *et al*.<sup>2</sup> The amount of rifampicin degraded in presence of isoniazid from novel dosage form was less than 0.21% at the end of 120 m (Fig. 4). The probable reason for this behavior could be enteric effect of HP-55S preventing isoniazid release in presence of rifampicin in acidic media (pH 1.5). The results thus underline the fact that minimization of contact between rifampicin and isoniazid results in less degradation of rifampicin. Enteric coating of isoniazid, therefore, is justified. It is worthwhile to note that isoniazid is well absorbed from the gastrointestinal track.<sup>3</sup>

#### CONCLUSION

Minimization of contact between rifampicin and isoniazid and sustained release of rifampicin can alleviate the degradation of rifampicin to a certain extent from the novel dosage form.

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Batch code	% weight gain	Time required to fracture HP-55s coat (m)	
		рН 1.5 (0.1 N НС1)	pH 6.8 phosphate buffer
A1	3	30	-
A2	6	70	-
A3	9	> 120	7

Table No.1 – Results of enteric test of batches A1-A3

Fig.1 Schematic diagram of novel dosage form of rifampicin and isoniazid



Fig.2 Schematic diagram of modified dissolution apparatus (not to scale)







Fig.4 Comparative in vitro drug degradation of rifampicin in presence of isoniazid



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