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In vitro evaluation of food effect on the bioavailability of rifampicin from antituberculosis fixed dose combination formulations

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Abstract

Rifampicin is one of the major first line anti-tuberculosis drugs used in the therapy of tuberculosis. In literature, there are conflicting reports regarding effect of food on the bioavailability of rifampicin. In vitro, effect of food on the bioavailability can be studied by simulating in vivo conditions in dissolution fluid hence, to understand the variable effect of food on rifampicin release, dissolution studies were done by simulating in vivo conditions after meal intake. In this study, we assessed the effect of hydrodynamic stress in presence of food and meal composition on two rifampicin containing fixed dose combination formulations by carrying out dissolution at different agitation rates (simulation of fasted and fed state) as well as in the presence of different percentage of oil (fatty food). Agitation intensity as well as presence of oil did not had any influence on rifampicin release from formulation A. This formulation had shown excellent release characteristics at all the conditions studied. Whereas, formulation B showed agitation rate dependent release and also release was affected in presence of oil. Hence, it is concluded that food may not have any effect on the release of rifampicin from the formulation and subsequently on its bioavailability if the formulation has excellent release profile ($>85\%$ release in 10 min). Further, effect of food on the rifampicin release was a function of dosage form characteristics such as disintegration time and dissolution rate, which will subsequently affect the release behavior of a formulation in presence of food.

Keywords: Rifampicin; Bioavailability; Food effect; Dissolution

1. Introduction

Dissolution test is the most important test employed for in vitro evaluation of solid dosage forms. In order to predict the in vivo performance of drug products, dissolution tests should be designed so as to simulate the in vivo conditions [1]. One of the important factors affecting bioavailability of the drug is the effect of food that induces changes in the physiology of the gastrointestinal tract resulting in delayed gastric emptying, changes in pH or stimulation of bile flow. Effect of food on bioavailability and bioequivalence depend on physico-chemical (solubility) and pharmacokinetic (site, rate, and extent of absorption, first pass metabolism) properties of the drug and on the dissolution of the drug substance from the drug product [2]. The physiological changes induced by food can be studied in vitro by simulating these conditions e.g. reduced agitation intensity as an indication of low hydrodynamic flow around the dosage form in the human GI tract in presence of food [3] and addition of co-administered fluid/food components to dissolution medium to reflect the conditions in the stomach soon after meal intake [4].

Tuberculosis patients have to consume 2–4 g of drugs in the fasting state that reduces patient compliance because of the gastric irritation associated with it. There are conflicting reports in the literature regarding effect of food on the bioavailability of rifampicin. Some reports state that absorption of rifampicin was not affected by food [5], whereas several groups reported decreased rifampicin bioavailability in presence of food [6–10]. A possible explanation for variable absorption

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seen for rifampicin may be the alteration of the pH or gastrointestinal motility. One more study by Washington et al. has shown that increased fat present in the meal has no effect on the pharmacokinetic parameters of rifampicin [11] may be because of increased solubilization of rifampicin in fatty part of meal but at the same time viscosity of the medium increases thereby reducing the hydrodynamic flow around the dosage form affecting its release.

In the above context, present preliminary study was undertaken to systematically evaluate the effect of food on the release behavior of rifampicin from fixed dose combination (FDC) formulations of anti-tuberculosis drugs by simulating these conditions in dissolution medium.

2. Materials and methods

2.1. Materials

To study the influence of food on rifampicin release, two FDC formulations were taken; formulation A was three drug FDC while formulation B was four drug FDC tablet containing fixed ratio of drugs as recommended by World Health Organization (WHO). Formulation A composed of rifampicin 120, isoniazid 50 and pyrazinamide 300 mg whereas formulation B was composed of rifampicin 150, isoniazid 75, pyrazinamide 400 and ethambutol 275 mg. Three and four drugs FDC tablets were supplied by WHO and Svizera laboratories, Mumbai, respectively. Rifampicin, isoniazid, pyrazinamide and ethambutol were gift samples from Lupin laboratories limited (Mumbai, India). All other chemicals used were of analytical grade procured from Ranbaxy laboratories limited (S.A.S. Nagar, India) or E. Merck India limited (Mumbai, India). Sunflower oil used in the study was procured from Hindustan lever limited (Mumbai, India).

2.2. Instruments

Instruments used were Electrolab dissolution tester (Mumbai, India), Beckman DU 640i Spectrophotometer (Fullerton, CA, USA), Mettler electronic balance AG 245 (Greifensce, Switzerland), Branson 3210 Sonicator (The Hague, The Netherlands), Brand Autopipettes (Germany), Hamilton Microlitre syringes (Bonaduz, Switzerland), REMI research centrifuge (Chandigarh, India).

2.3. Dissolution study at different agitation rates

Effect of agitation intensity on the rifampicin release as a function of hydrodynamic stress around the dosage form was studied by in vitro dissolution at different agitation rates (30, 50, 75 and 100 rpm, using USP Type II apparatus (paddle) at 37 ± 0.5 °C. The dissolution medium comprised of 900 ml of simulated gastric fluid without pepsin (Water:Solution Y, 850:50, Solution Y: 18 g NaCl + 63 ml concentrated HCl + water to make volume up to 500 ml). The 5 ml samples were withdrawn at 10, 20, 30 and 45 min with replacement by fresh dissolution medium. For each dissolution test, five tablets were used. The sixth vessel was used as reference vessel in which the pure drugs equivalent to amount present in the formulation were dissolved [12]. The dissolution parameters and methodology were kept constant in all the experiments except agitation rate. The samples collected at different time intervals were diluted with dissolution medium, analyzed immediately by spectrophotometer at 475 nm and % release of rifampicin was calculated with respect to the reference vessel [12].

2.4. Effect of oil

Effect of oil on the release profile of rifampicin from the formulation was studied by adding different percentage of oil (10, 20, 30, 35%) to the dissolution medium. This study was carried out in a similar manner at 75 rpm. In order to further study the effect of oil at higher agitation rate, the dissolution was also carried out at 200 rpm with 20% oil (equivalent to the amount of fats present in the normal Indian fatty meal). The samples were centrifuged at 7000 rpm for 10 min and bottom aqueous layer was analyzed at 475 nm. Percentage release of rifampicin was calculated with respect to the absorbance of reference vessel.

2.5. Data analysis

All the dissolutions profiles were evaluated statistically for their similarity by calculating similarity factor (f_2 value) taking 75 rpm as a reference. According to this method, the f_2 value between 50 and 100 suggests that two dissolution profiles are similar [13].

3. Results and discussion

3.1. Effect of agitation rate

Normally food delays the gastric emptying as motility is affected and to simulate this condition, the dissolution studies were carried out at different agitation rates (30, 50, 75 and 100 rpm). Low agitation intensity is an indication of reduced hydrodynamic flow around the dosage form in fed state and high agitation intensity simulates fasted state [3]. As is evident from Fig. 1, formulation A showed excellent release profiles releasing 90–100% rifampicin within 10 min, while formulation B

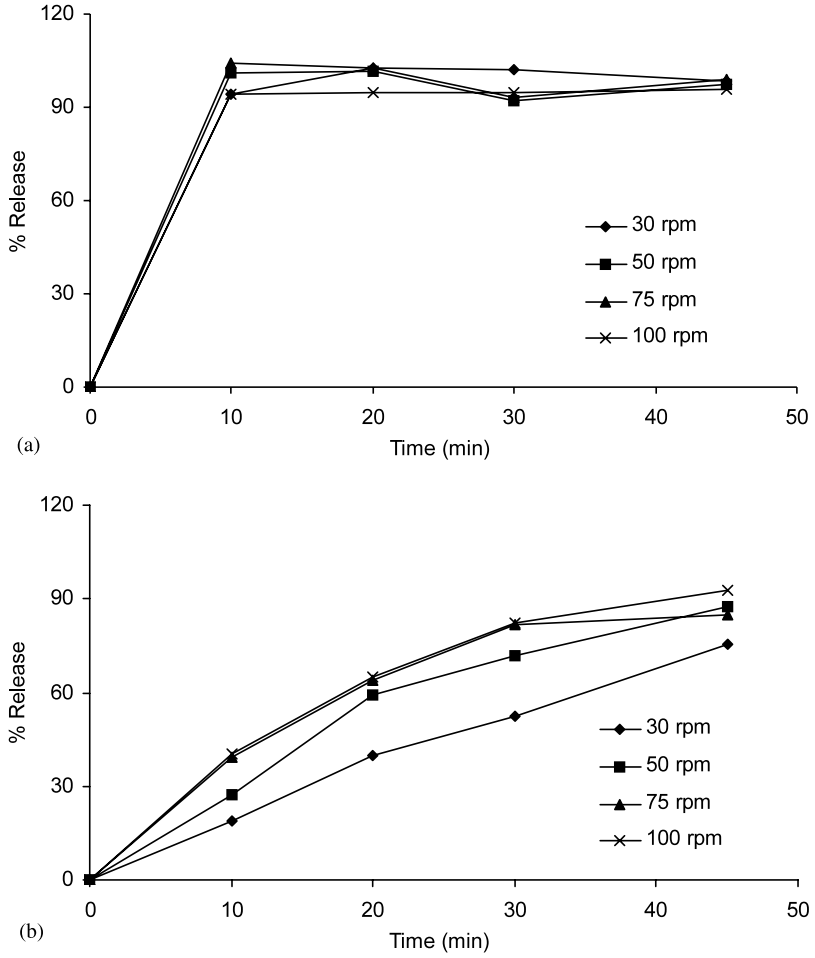

Fig. 1. Comparative dissolution profiles of rifampicin at different agitation intensities (a) Formulation A, (b) Formulation B.

did not show 100% release even after 45 min. Further, there was no statistically significant difference among the dissolution profiles of formulation A being f_2 values greater than 50 (Table 1). In addition, according to US FDA guidelines, when both test and reference products dissolve 85% or more of the label amount of the drug in 15 min, dissolution profiles may be accepted as similar without further mathematical evaluation [14]. Hence, based on this, dissolution profiles of formulation A at different agitation rates can be considered similar. On the other hand, formulation B, as can be seen from Fig. 1b, showed agitation dependant release at lower rpm i.e. dissolution rate of rifampicin decreased with decrease in agitation rate. But, the statistically significant difference was found only with 30 rpm when compared with dissolution rates at higher rpm. Despite of this difference in the dissolution profiles at different agitation rates, all the dissolution tests passed the USP dissolution criteria of not less that 75% release in 45 min at all the agitation rates [12].

Table 1
The f_2 values obtained after comparison of dissolution profiles of rifampicin containing FDC formulations at different agitation rates

	Formulation A	Formulation B
30 Vs 75 rpm	60.94	35.17
50 Vs 75 rpm	86.21	56.20
100 Vs 75 rpm	61.00	71.16

Two dissolution profiles were considered to be similar if similarity value f_2 is between 50 and 100 [13]. With USP type II dissolution apparatus (paddle), 75 rpm agitation intensity represents hydrodynamic stress at fasted conditions. Lower agitation intensity than this represents fed state [3]. Hence, 75 rpm was taken as reference to study effect of fed conditions.

3.2. Effect of oil

In the fed state, the lumenal composition of the stomach is highly dependent on the composition of the meal ingested. In order to simulate the in vivo conditions in the presence of fatty meal, a volume of oil reflecting the fat content of the meal can be added to dissolution medium [1] and hence dissolution were done in the presence of different percentage of oil added to dissolution medium. Fig. 2 shows the effect of presence of oil on the release of the rifampicin from FDC

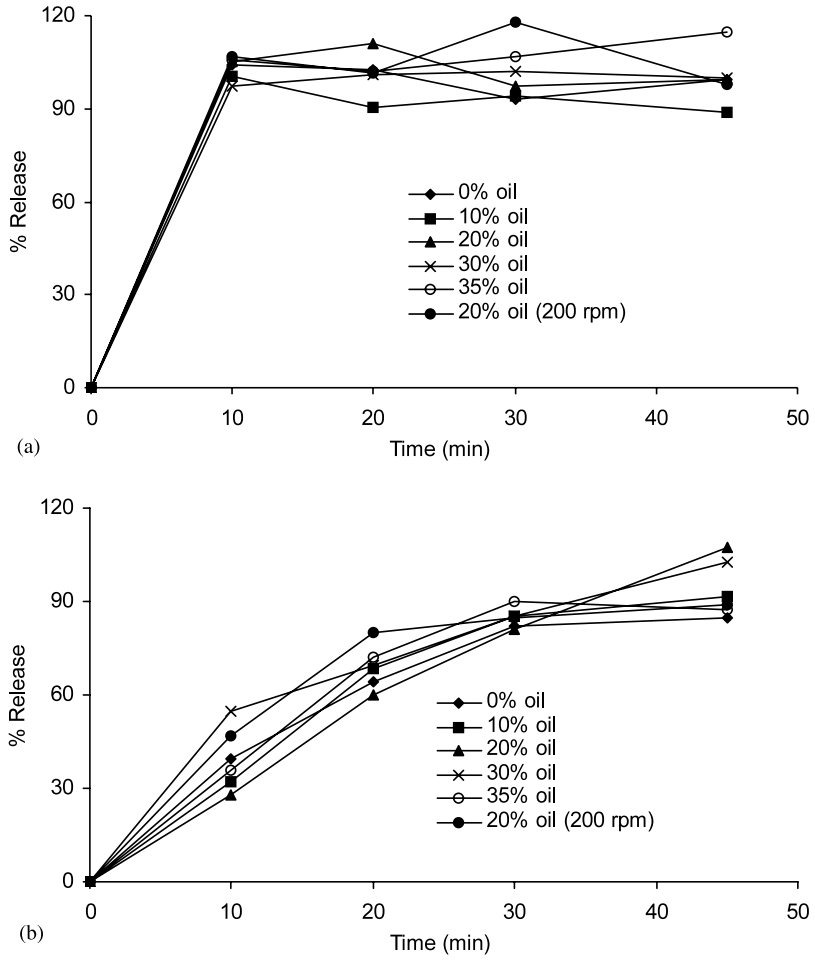

Fig. 2. Comparative dissolution profiles of rifampicin at 75 rpm using different percentage of oil in dissolution medium (a) Formulation A, (b) Formulation B.

formulations. For the purpose of comparison, dissolution profiles were evaluated by calculating f_2 values considering dissolution profile without oil at 75 rpm as a reference and listed in Table 2. Similar to agitation results, formulation A showed the maximum release within 10 min independent of oil content whereas in case of formulation B, release profile of rifampicin varied at different percentage of oil but no direct correlation between oil content and rifampicin release profile was observed in the f_2 values. Absence of any in vitro correlation with varying percentage of oil is in agreement with in vivo study in human volunteers where Washington et al. reported that increasing the fat content of meal did not alter the pharmacokinetic parameter of rifampicin compared to low fat meal when studied in healthy human volunteers [11].

Thus, agitation rate and percentage of oil did not have any effect on rifampicin release from formulation A as maximum drug was released within 10 min. However, in case of formulation B, the dissolution rate was decreased with the decrease in the agitation rate. This decrease in dissolution at lower rpm simulating the fed state may be due to increased disintegration time of the formulation as hydrodynamic stress experienced by the formulation is considered to be low [3]. These results are

Table 2
The f_2 values obtained after comparison of dissolution profiles of two FDC formulations containing rifampicin in the presence of different percentage of oil in the dissolution medium

	Formulation A	Formulation B
10% oil (75 rpm)	56.55	67.01
20% oil (75 rpm)	68.32	47.02
30% oil (75 rpm)	64.95	48.10
35% oil (75 rpm)	51.43	62.46
20% oil (200 rpm)	86.36	51.50

Note: All the f_2 values shown in the above table are calculated by considering the dissolution profile without oil at 75 rpm as a reference. Two dissolution profiles were considered to be similar if similarity value f_2 is between 50 and 100.

in agreement with the in vivo study by Peloquin et al. wherein they have studied the effect of high fat meal on the absorption of rifampicin on 14 volunteers. Their results indicate that compared to the fasting treatment food reduced the mean $C_{max.}$ (the highest drug level measured) by 36%, increased $T_{max.}$ (time to reach the highest concentration) by 103% but there was no effect on area under the curve (AUC) [10]. Similar results are reported by Haguland et al. that meal delayed absorption, but showed minor effects on $C_{max.}$ and AUC [15]. By comparing the results of in vitro dissolution study to that of in vivo studies, inference can be drawn that increased $T_{max.}$ is a result of increased disintegration and reduced dissolution rate of the formulation in fed state due to reduced hydrodynamic flow thereby affecting $C_{max.}$ and $T_{max.}$ values but complete absorption is found in the presence of food as AUC is unaffected.

This study indicate that the food can play an important role on the release of rifampicin but, this effect is a characteristics of the dosage form such as, disintegration time and dissolution rate of the formulation. In formulation A that exhibited very low disintegration time and excellent release profile ($>85\%$ in 10 min), rifampicin release was not affected by agitation rate and oil. On the other hand, with formulation B, agitation rate had effect on the release as the disintegration was longer resulting in poor release behaviour although the formulation passed the statutory requirement of 75% release in 45 min.

4. Conclusion

A very preliminary attempt was done to study the effect of food on the release behaviour of the rifampicin from the dosage form by simulating in vivo conditions in dissolution medium. This study constitutes a reliable baseline for evaluation of FDCs and effect of food on the bioavailability of rifampicin. To simulate the conditions in fed state, additional studies are required for validating the applicability of this methodology as a screening tool in rifampicin formulation development. After complete validation, this methodology will also serve as the guide for in vitro evaluation of food effect on product performance.

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