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Dissolution testing of marketed rifampicin containing fixed dose combination formulations using a new discriminative media: a post marketing retrospective study

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Currently recommended compendial dissolution methods for quality control of orally administered solid dosage forms of rifampicin containing formulations are not found to be able to forecast the *in vivo* performance. A recently proposed dissolution method of 0.01 N HCl at 50 rpm using paddle apparatus for screening was found to be more appropriate and able to predict the *in vivo* performance of those formulations. The objective of this investigation was to validate the new method of dissolution testing for solid dosage forms of rifampicin containing formulations using a basket apparatus and to compare it with the frequently recommended pharmacopeial method. In the present study the newly proposed dissolution condition (0.01 N HCl) was validated using six formulations of two, three and four drug combinations from two different manufacturers by basket method and compared with the widely recommended compendial medium. In this investigation, the appropriateness of the proposed methodology was confirmed by the dissolution results of the two FDC formulations (a two-drug and a four-drug combinations) that had previously passed the bioequivalence tests. It was found that the recommended dissolution medium of 0.01 N HCl can be used for screening of rifampicin containing formulations using both paddle and basket dissolution apparatus at 50 rpm and 100 rpm, respectively.

1. Introduction

After the declaration of tuberculosis (TB) as a global emergency by World Health Organization, TB therapy took a new dimension by the implementation of "directly observed treatment short course (DOTS)" using fixed dose combination (FDC) formulations of anti-TB drugs in national TB control programmes world wide. In spite of the potential advantages provided by FDCs with respect to patient compliance and preventing multi-drug resistance TB, the variable bioavailability of rifampicin from FDCs when compared to separate formulations pose a major hurdle for implementation of FDCs in DOTS (Panchagnula et al. 2004). Though the crucial decision of supplying FDCs only of proven bioavailability paved the way for access to quality formulations, the issue of batch-to-batch consistency with respect to their *in vivo* performance still exists. As reported by McIlleron et al. (2002), where the pharmacopeial dissolution methodology was unable to forecast a clinically significant lesser blood level from a rifampicin-only tablet, dissolution test meant for monitoring batch-to-batch consistency of the quality of formulations appears to be not working in case of rifampicin containing products. The lack of a predictive ability of the present pharmacopeial dissolution methodologies for rifampicin alone, 2, 3, and 4-drug FDCs (see Table 1) is attributed to a higher solubility of rifampicin in the recommended dissolution media and higher agitation intensities which would practically mask, if any changes are expected in the performance of formulation. In this process of optimizing proper dissolution conditions for rifampicin containing formulations, several studies were initiated and recently, dissolution in 0.01 N HCl and 6.8 pH phosphate buffer at 50 rpm in paddle apparatus with multiple sampling point has been recommended as a suitable dissolution condition (Agrawal and Panchagnula 2004a), where a good quality formulation is expected to pass in both the media. Thus, the objective of the present study was to evaluate FDCs (2, 3, and 4 drug combinations) from two different manufacturers using a basket (USP Type I) apparatus and compare with the recommended pharmacopeial method.

2. Investigations, results and discussions

In order to correlate the change in release profiles or dissolution profile of rifampicin with respect to the type of FDC, the present investigation utilizes all the three types of FDC formulations from two different leading manufactures (Table 2). Out of those six formulations, the bioequivalence studies for two formulations, a two-drug combination A2 and a four-drug combination C1 were conducted and were reported to pass the bioequivalence study when compared to that of loose combination at the same dose levels (Agrawal et al. 2004). Dissolution studies were also

Table 1: Specifications for dissolution methods recommended for rifampicin by United States Pharmacopoeia and its supplements

USP Year	23 1999	23 III Nov. 1999	24 Jan. 2000	24 I Jan. 2000	25 2002	26 2003
R Caps	0.1 N HCl; I; 50 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min	0.01 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min
R + H Caps		0.1 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min	0.01 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min	0.1 N HCl; I; 100 rpm; 45 min
R + H + Z				Official in USP 24 supplement 2	SGF TS; I; 100 rpm; 30 min*	SGF TS; I; 100 rpm; 30 min*
R + H + Z + E					Official in USP 25	10 mM pH 6.8 sodium phosphate buffer; II; 100 rpm; 45 min

* Release specification not less than (NLT) 80% in 30 min., elsewhere NLT 75% in 45 min. Abbreviations: HCl – hydrochloric acid, SGF – simulated gastric fluid, R – Rifampicin, H – Isoniazid, Z – Pyrazinamide, E – Ethambutol; Volume of the medium required is 900 ml; USP apparatus I- Basket, USP apparatus II- Paddle

USP23. United States Pharmacopoeial Convention, Inc., Rockville, MD. 1999. pp. 1382–1383.
USP23. United States Pharmacopoeial Convention, Inc., Rockville, MD. Suppl. 3. 1999 pp. 2976.
USP24. United States Pharmacopoeial Convention, Inc., Rockville, MD. 2000. pp. 1485–1487.
USP24. United States Pharmacopoeial Convention, Inc., Rockville, MD. Suppl. 2000. pp. 1786
USP25. United States Pharmacopoeial Convention, Inc., Rockville, MD. 2000. pp. 1531–1535.
USP26. United States Pharmacopoeial Convention, Inc., Rockville, MD. 2000. pp. 1640–1645.

conducted for those formulations (A2 and C1) as per USP recommendations at the time of biostudy, where A2 had passed the dissolution test requirement of 75% dissolution in 45 min in 0.1N HCl at 100 rpm in USP apparatus I. As there was no recommended pharmacopeial method for dissolution testing of four drugs FDC at the time of biostudy, dissolution test of FDC C1 was evaluated in simulated gastric fluid using USP apparatus II (method recommended for three drug FDC), where the formulation passed the performed dissolution test. Dissolution test was repeated for FDC C1 when four-drug FDC became official in USP and it was found that the formulation passed the test requirements (NLT 75% release in 45 min using pH 6.8 sodium phosphate buffer at agitation intensity of 100 rpm in USP apparatus II) (Agrawal and Panchagnula 2004a). McIlleron et al. (2002) reported that the present dissolution methodology of using 0.1 N HCl as dissolution medium for screening the rifampicin containing formulations may not be appropriate. According to the biopharmaceutic properties of rifampicin and the predictions of bioavailability using solubility values at different pH and permeability values from stomach, rifampicin could be absorbed 100% pH 1 (i.e., 0.1 N HCl) and 80–90% at pH 2 (0.01N HCl, the actual gastric conditions) (Amidon et al. 1995, Agrawal and Panchagnula 2005). Despite the wide solubility difference at 0.1 and 0.01 N HCl, rifampicin could be absorbed rapidly from both the conditions. Hence, the objective of this investigation was to study the dissolution profiles of different types of marketed FDC formulations at both the conditions of 0.1 N and 0.01 N HCl and recommend the suitable discriminative medium for dissolution of rifampicin containing FDC formulations.

Thus, in the present study, formulations were subjected to both the conditions as recommend by USP and Ashokraj et al. (2004) at various hydrodynamic conditions in USP apparatus I in order to determine the optimum dissolution

Table 2: Details of formulations subjected for dissolution studies

Type of fixed dose combination	Formulations	
	Manufacturer I	Manufacturer II
Two drug (R + H)	Formulation A1	Formulation A2
Three drug (R + H + Z)	Formulation B1	Formulation B2
Four drug (R + H + Z + E)	Formulation C1	Formulation C2

Abbreviation: R – rifampicin, H – isoniazid, Z – pyrazinamide and E – ethambutol hydrochloride

Note: Formulations A2 and C1 corresponds to the fixed dose combinations used in study B and F respectively as mentioned by Agrawal et al. (2004), which passed the bioequivalence trial compared to separate formulations as reference at the same dose levels. Fromulations A1, B1 and C1 were acquired from Macleods Pharmaceuticals Ltd, Mumbai whereas formulations A2, B2 and C2 were from Lupin Laboratories Ltd, Mumbai

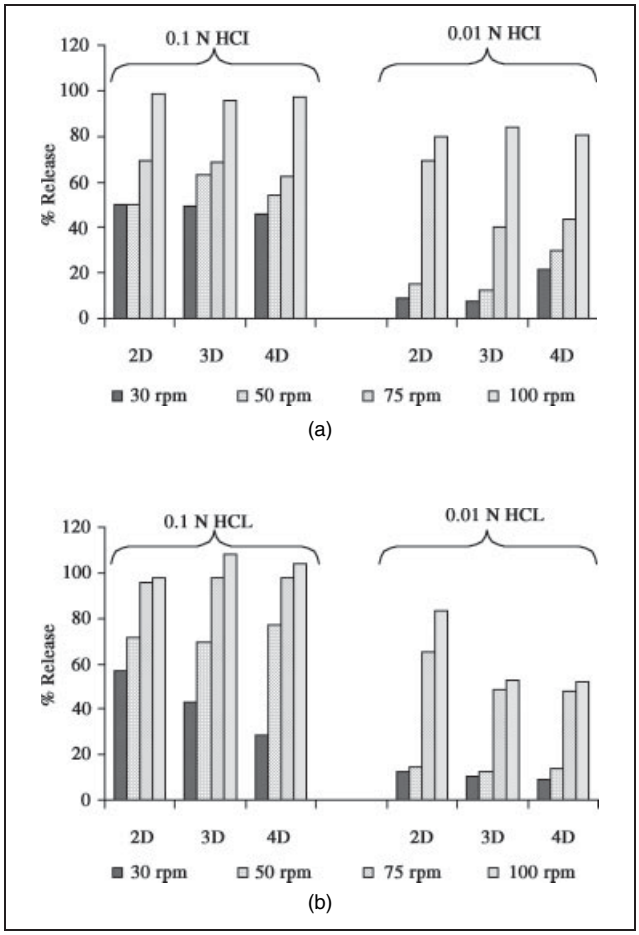

Fig.: Extent of rifampicin release in 45 min from various two (2D), three (3D) and four (4D) drug fixed dose combination formulations in two different media (0.1 N HCl and 0.01 N HCl) from two different manufacturers (a) manufacturer I and (b) manufacturer II at 30, 50, 75 and 100 rpm as agitation speed in USP apparatus I.
Note: All the values are mean of four determinations and the standard deviation in all the cases was found to be less than 10%

conditions by basket method and validate 0.01 N HCl as appropriate dissolution medium for rifampicin containing formulations. Since the pharmacopeial and the current method utilize both USP apparatus I and II, for different formulations (see Table 1) for the sake of uniformity and comparison in all the cases only the basket method was employed.

Before conducting the dissolution studies, drug content of all the formulations was determined and found to be within the pharmacopeial limits of 95–105% of the label claim for rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride (data not shown). With respect to dissolution, all the formulations were passing (from both the manufacturer) in 0.1 N HCl at 100 rpm as can be observed from the Fig. On the other hand none of the product fulfills the required dissolution test criteria of 75% in 45 min at 30 and 50 rpm even in 0.1 N HCl, in which rifampicin has a maximum solubility. The agitation intensity of 30 or 50 rpm does not reflect the appropriate hydrodynamic stress experienced by formulation in stomach. These results are in accord to the widely accepted general rule that an appropriate agitation condition in the basket method is 100 rpm.

A similar trend was observed in 0.01 N HCl also with no difference in dissolution at 30, 50 and 75 rpm as well as less extent of release, but an equal extent at 100 rpm, for all the three of the formulations from manufacturer I. With respect to rate of release (Table 3), formulations from manufacturer I in different media had shown to have a significant delay in release for four drug combinations in both the media, when a fast release followed by a delay in release can be observed for two and three drug formulations. Further these results are in good agreement with our previous claim of better predictability of 0.01 N HCl as dissolution medium for differentiating a good formulation from a bad one where formulation C1 showed ~90% of dissolution.

On the other hand products from manufacturer II showed an entirely different trend with respect to both rate and extent of release especially at 30 rpm and in 0.01 N HCl. A significant difference in extent of release was observed for all two, three and four drug combinations in 0.1 N HCl as well as 0.01 N HCl at all agitation intensities. Surprisingly the three drug and four drug formulations failed in 0.01 N HCl even at 100 rpm (only 50% drug release). The rate of release of these formulations were found to follow a similar trend to that of manufacturer I with an initial burst release and followed by a decrease in rate of release. Since an anticipated result was obtained in case of A2 and C1 where the formulations are passing the bioequivalence test as well as the dissolution test under the currently recommended conditions, it could be reasonably concluded that the other two formulations from manufacturer II may tend to release less amount of rifampicin and affect the ultimate bioavailability, which could be ascribed to appropriate raw material and manufacturing process (Agrawal and Panchagnula 2004a).

Dissolution is a prerequisite for oral drug absorption thereby reflecting the product performance. However, its relation to bioavailability of rifampicin from solid oral dosage forms is ill defined, resulting in costly bioequivalence tests to determine the quality of the formulations. In our lab, continuous efforts are being made to develop dissolution tests as a surrogate for *in vivo* bioequivalence trials. Earlier results published from our laboratory indicate good *in vivo* correlation with dissolution at 0.01 N HCl at 50 rpm using paddle apparatus (Agrawal and Panchagnula 2004a). In this investigation dissolution studies were carried out with a basket apparatus that is frequently used for capsule formulations and also a recommended method for three-drug FDC formulation.

The newly proposed dissolution conditions of 0.01 N HCl at 50 rpm by paddle method was found to be well applicable to basket method at 100 rpm. Out of six marketed formulation from different manufacturers across three different combinations four formulations were found to pass the dissolution test in which two were previously found to pass the bioequivalence test compared to separate formulations. Thus, it can be concluded from the present study that the currently recommended dissolution medium of 0.01 N HCl can be used for screening of rifampicin containing formulations using the paddle and basket method at 50 rpm and 100 rpm respectively and being able to differentiate between good and bad formulations.

3. Experimental

3.1. Materials and reagents

Rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride were supplied as *gratis* sample by Lupin laboratories, India as pure drugs. All other chemicals and reagents are either HPLC or analytical grade.

Table 3: Time of 30%, 50%, 80% parameters indicating the rate of drug release

Parameters	RPM	Manufacturer I						Manufacturer II					
		0.1 N HCl			0.01 N HCl			0.1 N HCl			0.01 N HCl		
		A1	B1	C1	A1	B1	C1	A2	B2	C2	A2	B2	C2
$t_{30\%}$	30	17.5	10.0	38.2	–	–	–	17.5	18.0	45	–	–	–
	50	9.6	10	22	–	–	–	6.8	13.8	13.8	–	–	–
	75	7.5	8.5	15.5	6	8	30	6	7	7	8	15.3	17
	100	4	6	10	4	6.2	6.2	3.8	4.5	4.5	6.2	10	13.2
$t_{50\%}$	30	45	36	–	–	–	–	34	–	–	–	–	–
	50	45	30	38	–	–	–	16.4	26	26	–	–	–
	75	18	21	22.5	10	21.3	–	15	12.3	12.3	21.3	–	–
	100	6.5	9.5	19.5	6.5	10.8	10.7	6	7.2	7.2	10.8	25.2	38.5
$t_{80\%}$	30	–	–	–	–	–	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–	–	–	–	–	–
	75	–	45	44	–	–	–	29.2	25	25	–	–	–
	100	10	26	30.5	–	29.6	26	9.8	16	16	29.6	–	–

Note:
1. Agitation intensity is given in revolutions per minute (RPM), in different dissolution medium (0.1 N HCl and 0.01 N HCl), for two (A), three (B) and four (C) drug combinations separately from manufacturer I and manufacturer II.
2. $t_{30\%}$, $t_{50\%}$ and $t_{80\%}$ are time taken for 30%, 50% and 80% of dissolution and the values are given in minutes.

3.2. Instruments

The HPLC system (Milford, MA, USA) consisting of two 515 pumps, 717 plus autosampler and 2487 dual wavelength detector, and UV-visible spectrophotometer (Beckman DU® 640i, Fullerton, Canada) was used for analysis. MILLENNIUM (version 2.1) software was used for acquisition and processing of data in HPLC. For dissolution studies Electrolab tablet dissolution tester (USP XXII, Mumbai, India) was used.

3.3. Assay

All the marketed samples were evaluated for drug content by various methods described in the literature. Colorimetric method of analysis at 475 nm for rifampicin, as recommended in the United States Pharmacopoeia (USP), was previously proved to provide accurate results to that of HPLC. Thus rifampicin was quantified by UV-spectrophotometer (Agrawal and Panchagnula 2004b), where as ethambutol hydrochloride was determined by the method described in USP 24 (2000). Isoniazid and pyrazinamide were determined by a HPLC method (Agrawal and Panchagnula 2001), using methanol, water, perchloric acid (70%) and tetra butyl ammonium hydroxide (40%) (2 : 8 : 0.005 : 0.0025), as mobile phase composition in reverse phased Spherisorb C_8 (250 X 4.6 mm i.d., 4 μm) column at the flow rate of 1 ml/min and detection wavelength was 267 nm.

3.4. Dissolution studies

Dissolution studies were performed using USP apparatus I in the pre-equilibrated medium (0.1 N HCl and 0.01 N HCl, 900 ml) at 37 ± 0.5 °C and various agitation intensities of 30, 50, 75 and 100 rpm. Five ml samples were withdrawn at regular time intervals with replacement of dissolution medium, suitably diluted and analysed by appropriate analytical methodologies as described above. Since rifampicin degrades at lower pH of 1–2, reference vessel method, as recommended in USP 26 (2003), was adopted to calculate the percent drug released. A reference vessel contained pure drugs equivalent to the amount present in the formulation dissolved in corresponding dissolution medium. Dissolution profiles were further analysed for extent (percent dissolution in 45 min) and rate (time to release 30%, 50% and 80% of rifampicin) of release for rifampicin.

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