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International Journal of Pharmaceutics 287 (2004) 97–112
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Dissolution test as a surrogate for quality evaluation of rifampicin containing fixed dose combination formulations

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Received 1 May 2004; received in revised form 25 August 2004; accepted 5 September 2004

Abstract

The present investigation was aimed at developing a dissolution methodology to predict in vivo performance of rifampicin containing fixed dose combination (FDC) products. Six FDC formulations were used in this study, of which four had passed bioequivalence while two failed. Dissolution studies were conducted at agitation intensity of 30–100 rpm as a measure of hydrodynamic stress and at pH media corresponding to gastric and intestinal conditions.

Formulations showed variable dissolution at different conditions and dissolution at 50 rpm was most sensitive and differentiated the release profiles of rifampicin under various pH conditions. It was possible to predict in vivo performance of rifampicin from FDCs when in vitro rate and extent of release at various pH was correlated with site, pH and concentration dependent absorption of rifampicin along with gastric emptying time. It was also seen that dissolution conditions recommended in USP for different types of FDCs were insensitive for the formulation changes. Based on this comprehensive evaluation, a decision tree is proposed which will act as a guideline for quality evaluation of FDC products and also will provide a fundamental knowledge for optimization of formulations failing in dissolution studies.

Keywords: Rifampicin; Fixed dose combination; Dissolution; Bioavailability; Bioequivalence; Tuberculosis

1. Introduction

Rifampicin is the key component of tuberculosis (TB) chemotherapy along with other first line anti-TB drugs and is used in both intensive and continuation phase for the treatment of all patient categories (Maher et al., 1997). For oral administration, rifampicin is present as separate formulation in the form of suspension, capsule and tablet or as FDC formulations combined with other anti-TB drugs like; isoniazid, pyrazinamide and ethambutol in a fixed ratio. Some of these tested formulations have shown varied plasma profiles and unacceptable bioavailability compared to corresponding separate formulations at the same dose

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doi:10.1016/j.ijpharm.2004.09.005

levels (Panchagnula and Agrawal, 2004). This variable bioavailability of rifampicin is considered as a major obstacle in the effective implementation of FDCs in national TB programs and thus successful treatment of the same (Blomberg et al., 2001; Blomberg and Fourie, 2003). Although dissolution testing is considered as an important tool for quality evaluation of the formulations, this holds poor in relation to rifampicin bioavailability. While rifampicin formulations with good dissolution properties appeared to be poorly absorbed, inverse relationship in context to rifampicin bioavailability is also reported (Acocella, 1989; Aspesi, 1989). Against the background of disparity between dissolution testing and bioavailability, World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) recommended use of FDCs with proven bioavailability of rifampicin (Anonymous, 1994).

Since the refinement of biopharmaceutic properties of oral bioavailability by means of 'Biopharmaceutic Classification System (BCS)', solubility and permeability are considered as fundamental properties in predicting the in vivo performance of oral drug products (Amidon et al., 1995) and has resulted in number of regulatory guidelines that reduce time consuming bioavailability studies and simplifies the drug approval process (FDA, 2000; Lobenberg and Amidon, 2000). Apart from rate and extent of drug release/dissolution, successful oral delivery of drug from immediate release (IR) solid dosage form is a function of effective permeability, stability in GI tract, interaction with concomitantly administered drugs, efflux and metabolism (Martinez and Amidon, 2002). With the increased understanding of the factors affecting dissolution and its influence on the absorption process it is possible to develop dissolution methodology to understand the performance of rifampicin containing formulations in vivo. Hence, a comprehensive investigation of rifampicin physicochemical properties, single dose pharmacokinetic studies and permeability characterization were done considering the physiologic variations of gastrointestinal (GI) tract (Agrawal and Panchagnula, 2004a) and are briefly explained (Table 1) here to understand the in vivo performance of rifampicin containing FDCs when coupled with dissolution process. Due to zwitter-ionic nature, rifampicin shows wide variation in the solubility at physiologic pH. Although it exhibits high solubility in the acidic media, in the gastric pH range of 1–3 solubility changes to 100 times from 125 to 1.2 mg/ml. At duodenal pH it is less soluble whereas at distal intestinal pH it is highly soluble. Effect of various factors revealed that rifampicin shows pH and site dependent permeability with lowest at gastric and highest at duodenal pH. Thus, rifampicin is a borderline

Table 1
Biopharmaceutic properties of rifampicin in different segments of GI tract influencing dissolution and absorption

GI segment	GI tract pH[a]	Transit time (min)	Surface area (m^2)	pH	Solubility (mg/ml)	Dose:solubility ratio[b] (ml)	P_{eff} (cm/s)	fa (%)	MAD (mg)
Stomach	1.2–3.0 (2.0)	90	0.11	1.40	125.5	5	0.02×10^{-4}	45	66
				2.36	11.4	52			
				3.00	1.15	521			
Duodenum	4.0–6.8 (4.5)	45	0.09	4.00	0.99	604	0.62×10^{-4}	100	559
				4.50	1.25	479			
				5.20	1.53	393			
Jejunum	6.0–7.0 (6.5)	180	60	6.00	1.65	364	0.24×10^{-4}	100	1484
Ileum	6.5–7.4 (6.8)	180	60	6.8	2.54	236	0.40×10^{-4}	100	2509
Colon	5.0–8.0 (7.4)	360	0.25	7.4	3.35	179	0.12×10^{-4}	100	1691
				8.0	5.44	110			

P_{eff} values of rifampicin from the small intestinal segments corresponds to highly permeable molecule ($>0.2 \times 10^{-4}$ cm/s).

[a] Figures in parentheses represent the mean pH of the GI segment and permeability values were determined at this pH.

[b] Dose solubility ratio should be less than 250 ml in the physiologic pH range to consider a drug as highly soluble. Fraction of dose absorbed (fa) and maximum absorbable dose (MAD) from different segments were calculated from mean effective permeability (P_{eff}) taking into consideration pH, transit time and solubility of rifampicin. MAD is the quantity of drug that could be absorbed if GI segment is saturated till its residence time and takes into consideration solubility, dose, volume and transit time of the GI tract segment.

Table 2
Dissolution test specifications for rifampicin from anti-TB formulations official in USP

	R capsule (rifampicin-only)	RH capsule (two drug FDC)	RHZ tablet (three drug FDC)	RHZE tablet (four drug FDC)
Assay limit	90–110%	90–130%	90–110%	90–110%
Dissolution medium	0.1N HCl 0.01N HCl[a]	0.1N HCl 0.01N HCl[a]	Simulated gastric fluid TS, without pepsin	10 mM pH 6.8 phosphate buffer
Dissolution volume	900 ml	900 ml	900 ml	900 ml
Apparatus	Basket	Basket	Basket	Paddle
rpm	100	100	100	100
Time	45 min	45 min	30 min	45 min
Q limit	75%	75%	80%	75%
Official in	USP 24, USP 25, USP 26	USP 24, USP 25, USP 26	USP 25, USP 26	USP 26

In USP 24 (2000) only rifampicin capsule and rifampicin plus isoniazid capsule were official where dissolution medium was 0.1N HCl. Three drug FDC became official in Supplement 2 to USP 24. But this monograph did not specify dissolution test conditions. Later dissolution was added to three drug FDC monograph in USP 25 (2002). Finally, four drug FDC became official in USP 26 (2003). In British Pharmacopoeia (BP, 2002) and Pharmacopoeia of India (IP, 1996) rifampicin-only capsule is official with 0.1N HCl as a recommended medium for dissolution. R, rifampicin; H, isoniazid; Z, pyrazinamide; E, ethambutol; FDC, fixed dose combination; TS, test solution; *Q*, percentage of the labeled amount to be released in dissolution within a specified time.

[a] In Supplement 1 to USP 24, dissolution medium for rifampicin and rifampicin plus isoniazid capsules was changed to 0.01N HCl that subsequently was again changed to 0.1N HCl in USP 25 (2002).

class II drug of BCS. It was observed that rifampicin is a highly permeable *p*-glycoprotein (*p*-gp) substrate and hence shows dose dependent absorption, which is apparent only at lower concentrations (Agrawal and Panchagnula, 2004a).

In case of rifampicin, existing compendial dissolution methodologies do not guarantee acceptable bioavailability from the FDC formulations of anti-TB drugs. Table 2 shows the dissolution test specifications for rifampicin from single or FDC formulations as recommended in United States Pharmacopeia (USP). As evident from Table 2, there is no uniformity in pH of dissolution media, agitation conditions, *Q*-values and dissolution time for different types of FDCs. In these dissolution media, solubility of rifampicin is in the range of 2.54–125 mg/ml. Further for four drug FDC, dissolution apparatus is paddle at 100 rpm having very high agitation intensity than is actually present in the GI tract around the dosage form. In addition, USP monographs for different types of FDCs differ for the specified (Table 2). Very high solubility in 0.1N HCl or SGF and high agitation intensity may likely to mask any variations (batch-to-batch quality control or effect of manufacturing and/or process variables) in the rifampicin containing dosage forms. Hence, in light of above discussion this investigation is aimed at determining a dissolution methodology that can be used as a surrogate marker for in vivo performance of rifampicin containing formulations.

2. Materials and methods

2.1. Materials

Rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride were gift samples from Lupin Laboratories Limited (Mumbai, India). All other reagents and chemicals used were of analytical grade or HPLC grade procured from Ranbaxy Laboratories Limited (S.A.S. Nagar, India), E. Merck India Limited (Mumbai, India) and Mallinckrodt (Kentucky, France). Polycarbonate filters were acquired from Millipore (Ireland). Freshly prepared de-ionized water was used for dissolution studies whereas ultra-pure water prepared by reverse osmosis and filtered through 0.45 μm membrane filter was used for HPLC analysis.

2.2. Instruments

For analysis of bioequivalence study samples, Waters HPLC system (Milford, MA, USA) consisting of two 515 pumps, 717 plus autosampler and 2487 dual λ absorbance detector was used. Millennium software

(version 3.05.01) was used for data acquisition and processing. All the dissolution studies were performed with Electrolab tablet dissolution tester (Mumbai, India) and samples were analyzed on Beckman DU® 640i spectrophotometer (Fullerton, CA, USA). Other instruments used include Thermo-Orion digital pH meter attached to glass electrode (Beverly, MA, USA), Elgastat (ELGA Ltd., Bucks, UK), electronic balance AG 245 (Greifensee, Switzerland), Branson 3210 sonicator (The Hague, The Netherlands), Maxi dry lyo from Heto (Allerod, Denmark), Biofuge primo from Heraeus (Hanau, Germany), Millipore syringe filtration assembly (Bangalore, India), Brand autopipettes from E. Merck (Mumbai, India) and microlitre syringes from Hamilton (Bonaduz, Switzerland).

2.3. Formulations

Details of the formulations used for bioequivalence studies such as FDC and corresponding separate formulations, dosage form, type of FDC, its strength etc are given in Table 3. All the FDC formulations were coded as FDC C to FDC H whereas separate formulations were coded as SEP C to SEP H. The separate 'rifampicin-only' formulations were either hard gelatin capsules or sugar coated tablets whereas FDC formulations were film coated tablets. FDC C-G were four drug and FDC H was three drug combination. Six FDC formulations were from three different manufacturers where FDC C and D, E and G, F and H corresponded to same manufacturer. Before conducting bioequivalence studies it was ensured that all the formulations are according to pharmaceutical specifications and passed quality control tests with respect to uniformity of tablet weight, content of active drug substance and dissolution (USP, 2003). Formulations were stored according to their recommended storage conditions given on the label and used before the expiry date for the planned dissolution studies.

2.4. Conduction of bioequivalence trials

All bioequivalence studies were designed as single-dose, two-treatment, two-period crossover experiment with 1-week washout period utilizing healthy volunteers. Details of these studies; such as formulation specifications, dose, protocol followed, number of volunteers and sampling points etc are given

Table 3
Details of the bioequivalence trials of FDC formulations vs. separate formulations of anti-TB drugs conducted at NIPER bioavailability center

Study code[a]	Dose[b] (mg)	FDC[c]	FDC strength[d] (mg)				Separate R formulations[e]	Formulations[f]		Number of Volunteers[g]	Sampling time[h] (h)
			R	H	Z	E		FDC	Separate		
Study C	450	Tablet/4 drug	150	75	400	275	Capsule: 450 mg	3	4	13	36
Study D	450	Tablet/4 drug	150	75	400	275	Capsule: 450 mg	3	4	14	24
Study E	450	Tablet/4 drug	150	75	400	275	Tablet: 450 mg	3	4	13	24
Study F	450	Tablet/4 drug	225	150	750	400	Capsule: 450 mg	2	5	14	24
Study G	600	Tablet/4 drug	150	75	400	275	Capsule: 300 mg	4	7	22	24
Study H	600	Tablet/3 drug	150	75	400	–	Capsule: 300 mg	4	5	19	24

Before initiation of each study, in vitro quality control tests (weight variation, assay and dissolution) of all the formulations were done in order to judge the pharmacopeial compliance of the formulations. All the formulations passed in vitro quality control tests. FDC, fixed dose combination; R, rifampicin; H, isoniazid; Z, pyrazinamide; E, ethambutol; TB, tuberculosis.

[a] In the text, FDC or separate formulations used in the particular study are denoted by FDC or SEP suffixed by study code.

[b] Dose used in the trials was for Indian population (450 mg) as well as for International population (600 mg).

[c] Bioequivalence trials were conducted using different types of FDC such as 3 or 4 drugs FDC which are used in various phases of TB treatment according to patient category. All the FDC tablets were film coated.

[d] Studies were conducted using WHO recommended and non-recommended (FDC F) combinations.

[e] Separate formulations were either hard gelatin capsules or sugar coated tablet.

[f] The major advantage of FDC is to increase compliance by reducing number of formulations to be ingested. This can be appreciated from this column.

[g] Studies were conducted according to WHO recommended simplified protocol (19–22 volunteers) and by DCGI protocol (12–14 volunteers).

[h] Sampling points used were according to WHO protocol (8 h) and Indian regulatory protocol (24 h). As the half life of pyrazinamide is 8–10 h, one study for FDC C was conducted upto 36 h. Two trials, FDC G and FDC H were conducted as volunteer requirement of WHO protocol and sampling time requirement of DCGI protocol for comparison purpose.

in Table 3. After the approval of ethical committee, studies were conducted at NIPER bioavailability center and described elsewhere in detail (Agrawal et al., 2002a, 2002b; Panchagnula et al., 1999a, 1999b, 2000). Formulations were considered bioequivalent for rifampicin if estimates of bioequivalence fall between 0.8 and 1.25 of the corresponding separate formulations (Fourie et al., 1999; Panchagnula et al., 1999a).

2.5. Dissolution methodology

Effect of hydrodynamic stress on the rifampicin dissolution from FDCs were studied by conducting dissolution studies at different agitation rates of 30, 50, 75 and 100 rpm corresponding to low and high hydrodynamic stress experienced by dosage form (Shah et al., 1992; Scholz et al., 2002), whereas release at gastric and intestinal pH were studied by performing dissolution studies at three different dissolution media such as 0.1N HCl, 0.01N HCl and 6.8 pH phosphate buffer (Dressman et al., 1998). As the dissolution conditions were different for FDCs as per USP, these tests were conducted using six stage dissolution apparatus with USP apparatus II (paddle) specifications at $37 \pm 0.5\,^{\circ}C$ using 900 ml of dissolution fluid (Table 2). For each dissolution test five tablets were used and sixth vessel was used as a reference vessel in which pure drugs equivalent to amount present in the formulation were dissolved (USP, 2003). At 10, 20, 30 and 45 min time intervals, 5 ml samples were withdrawn with replacement, diluted with dissolution medium and analyzed immediately by colorimetry at 475 nm without the interference of other anti-tuberculosis drugs (Agrawal and Panchagnula, 2004b). Evaluation of dissolution profile was done by calculating dissolution efficiency (DE) in 45 min which is defined as area under the dissolution curve upto a certain time, expressed as a percentage of area of the rectangle described by 100% dissolution at the same time (Banakar et al., 1992). To study the effect of dissolution conditions on the rifampicin release, %DE values were compared by multiple-pair wise ANOVA (Tukey test) at 95% confidence interval.

3. Results and discussion

3.1. Bioequivalence of rifampicin from FDCs versus separate formulations

As there is no in vitro surrogate test to determine the quality of rifampicin containing dosage forms, WHO and IUATLD recommend the use of FDCs with proven bioavailability (Laing et al., 1999). Hence, quality of six FDC formulations was evaluated by bioequivalence testing conducted at NIPER bioavailability center. The pharmacokinetic parameters of rifampicin, such as

Table 4
Pharmacokinetic parameters and estimates of bioequivalence for rifampicin resulted from different bioequivalence trials

Code	Dose (mg)	AUC_{0-8} (μg h/ml)[a]		C_{max} (μg/ml)[a]		T_{max} (h)[a]		BE result	Estimates of BE[b]	
		FDC	Separate	FDC	Separate	FDC	Separate		AUC_{0-8}	C_{max}
FDC C	450	18.0 ± 8.2	19.4 ± 8.7	3.4 ± 1.6	3.9 ± 2.0	1.8 ± 0.8	1.8 ± 0.7	Failed	0.82–1.04	0.75–1.03[c]
FDC D	450	39.8 ± 8.2	29.6 ± 6.9	7.5 ± 1.4	5.6 ± 1.3	1.9 ± 0.6	2.6 ± 0.8	Failed	1.17–1.563	1.16–1.37[c]
FDC E	450	27.3 ± 5.6	26.1 ± 5.9	5.5 ± 1.6	5.4 ± 1.3	2.5 ± 0.9	2.5 ± 0.8	Passed	0.91–1.16	0.87–1.20
FDC F	450	31.8 ± 7.3	31.3 ± 7.6	7.1 ± 1.8	6.6 ± 1.6	2.0 ± 0.8	2.0 ± 0.7	Passed	0.89–1.16	0.92–1.24
FDC G	600	51.3 ± 10.8	57.6 ± 14.0	10.2 ± 2.4	11.6 ± 2.7	2.1 ± 1.0	2.3 ± 0.7	Passed	0.82–0.99	0.81–0.96
FDC H	600	48.1 ± 10.1	51.5 ± 9.2	9.6 ± 2.1	10.5 ± 2.1	1.7 ± 0.9	2.1 ± 0.9	Passed	0.88–0.99	0.84–0.99

Bioequivalence estimates of only primary parameters, viz. AUC and C_{max} are given in the text. T_{max} is the secondary parameter for the estimation of bioequivalence and is often affected by sampling points. However, it can be seen from T_{max} column that T_{max} of both combined and separate formulations is around 2 h. Bioequivalence studies where dose was 600 mg have shown C_{max} of around 10 μg/ml with the corresponding higher AUC whereas the trials with 450 mg dose have shown considerably lower C_{max} and AUC. After normalization of AUC for 600 mg dose, there was statistically significant difference in 450 mg dose group and 600 mg dose group, indicating the dose proportionality in the absorption of rifampicin. All the above FDCs were bioequivalent for isoniazid and pyrazinamide when compared to separate formulations at the same dose levels. FDC, fixed dose combination; BE, bioequivalence.

[a] All the values are given as mean ± S.D. Sample size (n) is given in Table 1.

[b] Statistical evaluation was done by Hauschke analysis at 90% confidence interval by 2 one-sided test.

[c] Outside the limits of bioequivalence (bioequivalence limit: 0.80–1.25).

AUC_{0-8}, C_{max} and T_{max} obtained from six studies and its primary estimates of bioequivalence are given in Table 4. Out of six formulations tested, four combined formulations FDC E to H have passed the bioequivalence test. On the other hand, C_{max} of rifampicin from FDC C was reduced in comparison to separate formulation. Interestingly, FDC D was suprabioavailable to its separate formulation with 35% increase in AUC as well as C_{max} and resulted in the highest blood levels from all the formulations given at 450 mg dose. Thus, depending on the plasma concentration and bioequivalence test result, these formulations fall in either good quality (bioequivalence test passed) or bad quality (bioequivalence test failed with reduced or increased bioavailability) FDCs. As these formulations have shown wide variations in the plasma concentration-time profiles and represent products from three different manufacturers, these FDCs were used for the development of dissolution methodology.

3.2. Dissolution study

Dissolution is a dynamic process strongly dependent on both composition of medium and hydrodynamics. As the luminal environment in the proximal GI tract varies considerably, it is worth considering the use of several variables to arrive at a complete picture of how an IR dosage form releases its active component under various GI conditions (Martinez and Amidon, 2002). Dissolution studies were done at different agitation speeds and also at various pH (0.01N HCl, 0.1N HCl and 6.8 pH buffer) as rifampicin shows pH dependent solubility corresponding to physiologic pH of the GI tract segments. In all the dissolution profiles, coefficient of variation was less than 10 and 5% for first sampling and subsequent sample points, respectively. Under all conditions, formulations from same manufacturers have shown similar trend in dissolution profiles and hence dissolution profiles of two FDCs from the same manufactures are shown in one figure. Dissolution profiles of FDC C and D, E and G, and F and H are shown in Figs. 1–3, respectively (%DE values are listed in Table 5).

Table 5
Dissolution efficiency (%DE) of rifampicin as a function of agitation intensity and dissolution medium from six different FDC formulations

Formulation	Dissolution medium	%DE			
		30 rpm	50 rpm	75 rpm	100 rpm
FDC C	0.1N HCl	55.9	73.9	71.6	72.8
	0.01N HCl	36.4	52.1	54.1	56.7
	6.8 pH buffer	42.3	66.8	71.6	74.2
FDC D	0.1N HCl	54.8	76.2	78.9	79.0
	0.01N HCl	40.3	58.4	59.3	59.2
	6.8 pH buffer	40.8	80.7	82.7	84.1
FDC E	0.1N HCl	43.5	68.9	85.5	84.6
	0.01N HCl	29.4	73.5	85.6	86.7
	6.8 pH buffer	18.8	39.8	51.4	51.5
FDC F	0.1N HCl	60.1	79.8	84.1	82.5
	0.01N HCl	56.7	78.1	78.9	83.0
	6.8 pH buffer	46.8	66.9	71.3	72.0
FDC G	0.1N HCl	37.4	55.6	79.5	83.5
	0.01N HCl	34.3	60.0	74.7	77.0
	6.8 Ph buffer	24.6	41.6	54.5	56.5
FDC H	0.1N HCl	65.2	83.0	83.8	84.6
	0.01N HCl	48.8	66.0	66.3	66.3
	6.8 pH buffer	35.7	61.3	63.3	65.1

DE values are mean of five tablets with S.D. was less than 5% in all the cases. Dissolution efficiency depends on the performance of formulation both in terms of rate and extent of release and is defined as area under the dissolution curve upto a certain time, expressed as a percentage of area of the rectangle described by 100% dissolution at the same time. However, it has limitation that the contribution of rate or extent cannot be determined.

3.2.1. Effect of agitation intensity

For in vitro quality determination of rifampicin containing formulations, dissolution method should be sensitive enough to differentiate manufacturing/process variables and should not be under or over discriminative. The proper medium and appropriate rotational speed of the paddle or basket are of great importance in assuring that the test procedure is useful and discriminatory. Hence, changing the agitation speed altered the measured dissolution rates and affected the ability of an in vitro test to distinguish in vivo performance for both IR and extended release (ER) formulations in several studies (Scholz et al., 2002; Abrahamsson et al., 1999; Katori et al., 1995; Shah et al., 1992).

In two formulations FDC C and D, agitation dependent release was seen only upto 50 rpm. At 50 rpm, both the formulations have shown good release in 0.1N HCl and 6.8 pH phosphate buffer with maximum release by 20 min (Fig. 1; also see Table 5). During dissolution of FDC C in 0.01N HCl, it was observed that large gran-

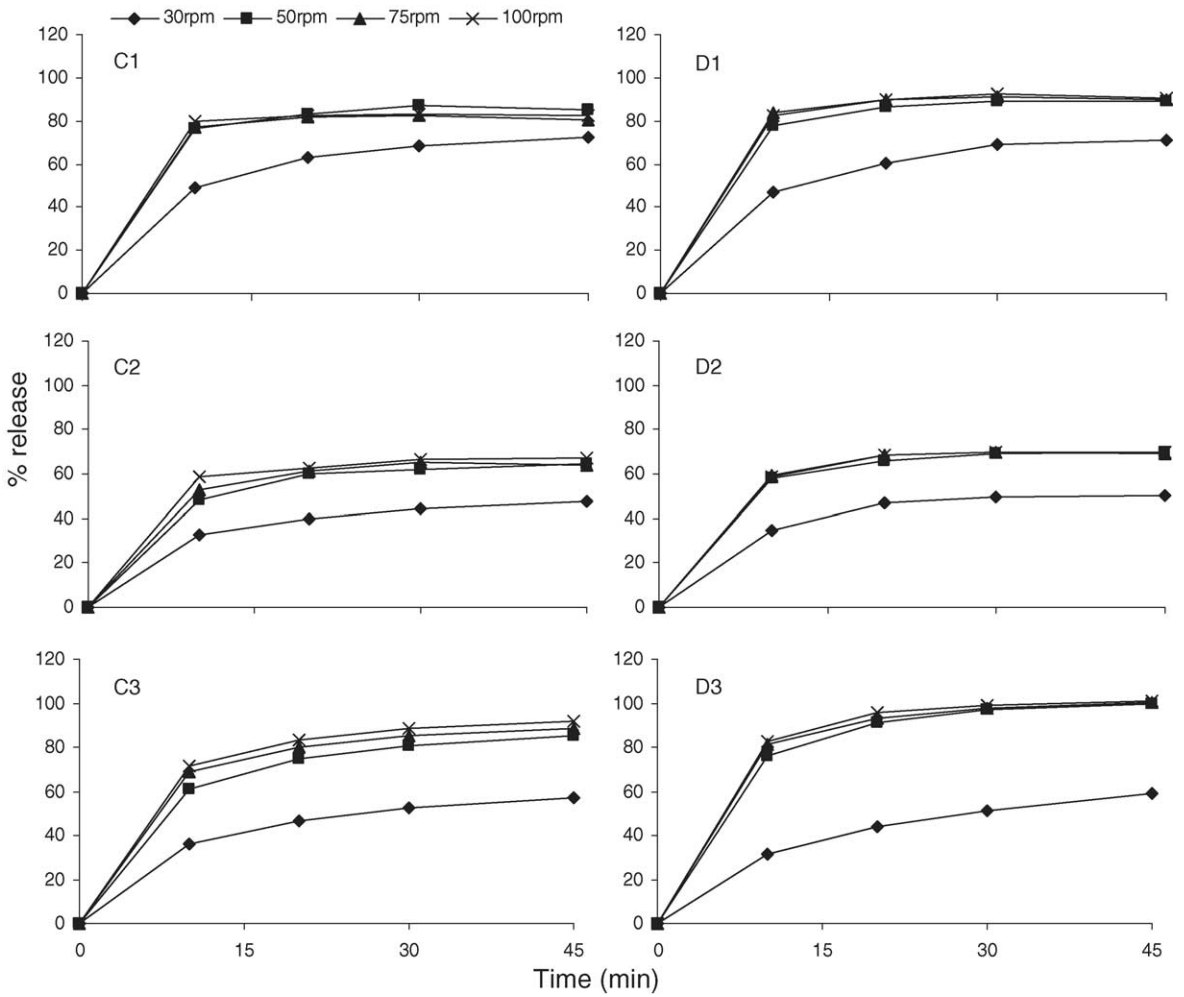

Fig. 1. Dissolution profiles of rifampicin from FDC C and FDC D tablets as a function of agitation intensity and pH of the dissolution medium (C and D represent FDC C and D whereas suffix 1, 2 and 3 represent dissolution in 0.1N HCl, 0.01N HCl and 6.8 pH phosphate buffer, respectively). Dissolution profiles are represented as mean of five tablets, S.D. in first sampling point was less than 10% and for subsequent samples it was less than 5%. These two formulations have shown similar behaviour in in vitro dissolution studies, however, FDC C was below the lower limit and FDC D was above the upper limit of bioequivalence. FDC C and D exhibited agitation dependent release upto 50 rpm. In contrast to rifampicin solubility, FDC C and D have shown reduced dissolution at 0.01N HCl and increased dissolution at 6.8 pH buffer.

ules in the size range of 500–1500 μm (particle size measured after decanting dissolution fluid at the end of the test) were settled at the bottom of the dissolution vessel and increase in agitation intensity had no effect on the dissolution of these granules. In case of FDC D, fine granules (200–500 μm) were observed which remained un-dissolved irrespective of higher agitation intensity.

FDC E and G have shown agitation dependent release upto 75 rpm, with no difference in percentage release at 75 and 100 rpm (Fig. 2). As seen in Fig. 3, FDC F and H exhibited excellent release (not less than 80% in 20 min) in all media at agitation speed of 50 rpm and hence effect of increased agitation speed cannot be expected beyond 50 rpm.

As expected, all the formulations have shown lowest release at 30 rpm in all the three media that is significantly different to that of 50 rpm (Table 5). At lower speed, hydrodynamics in the dissolution vessel is explained by laminar flow conditions characterized by

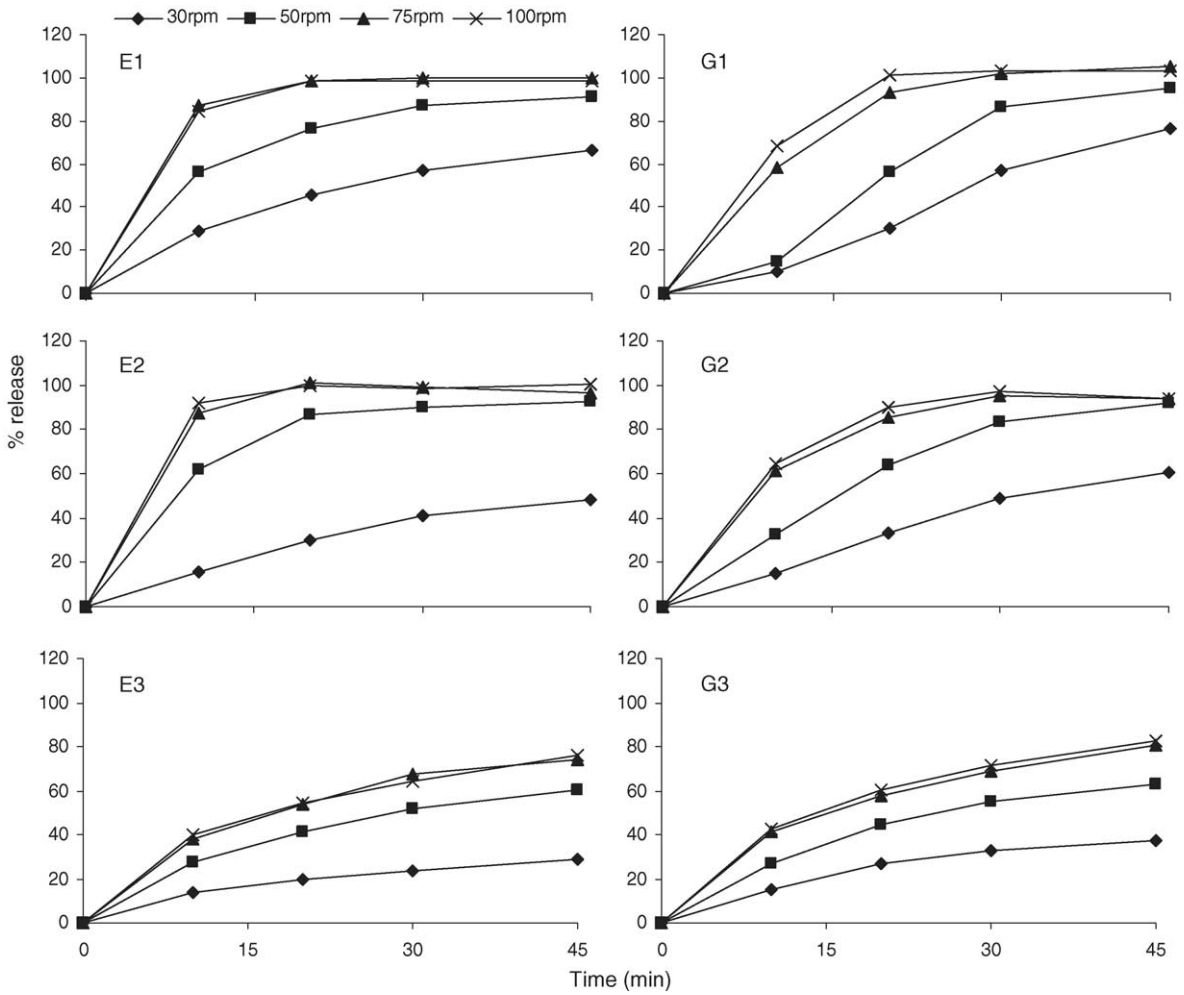

Fig. 2. Dissolution profiles of rifampicin from FDC E and FDC G tablets as a function of agitation intensity and pH of the dissolution medium (E and G represent FDC E and G whereas suffix 1, 2 and 3 represent dissolution in 0.1N HCl, 0.01N HCl and 6.8 pH phosphate buffer, respectively). Dissolution profiles are represented as mean of five tablets, S.D. in first sampling point was less than 10% and for subsequent samples it was less than 5%. FDC E and G have resulted in appreciable blood levels and passed the bioequivalence test when compared with separate formulations at the same dose levels. These two formulations have shown agitation dependent release upto 100 rpm.

segregation that compromises robustness of the test and makes it more vulnerable to variability with respect to sample location (Kukura et al., 2003; Healy et al., 2002). As high variation in rifampicin release was found at 30 rpm, it was neglected for further correlations. On the other hand, as evident from comparable D_{45}, $T_{50\%}$ and %DE between 75 and 100 rpm (Table 5; both D_{45}, $T_{50\%}$ results not shown), no significant difference was found between releases at these two intensities. For dissolution of four drug FDC tablet, USP 26 specifies apparatus II at 100 rpm however at 6.8 pH buffer, maximum dissolution was seen at 75 rpm for FDC E and G, whereas other four FDCs did not show increase in dissolution beyond 50 rpm. Moreover, dissolution at high agitation rate of 100 rpm may not serve as a means of quality control for assuring batch-to-batch uniformity as the hydrodynamic flow around the dosage form in the human GI tract could be extremely low and significant mixing of the material held in the fundus of the stomach does not take place (Shah et al.,

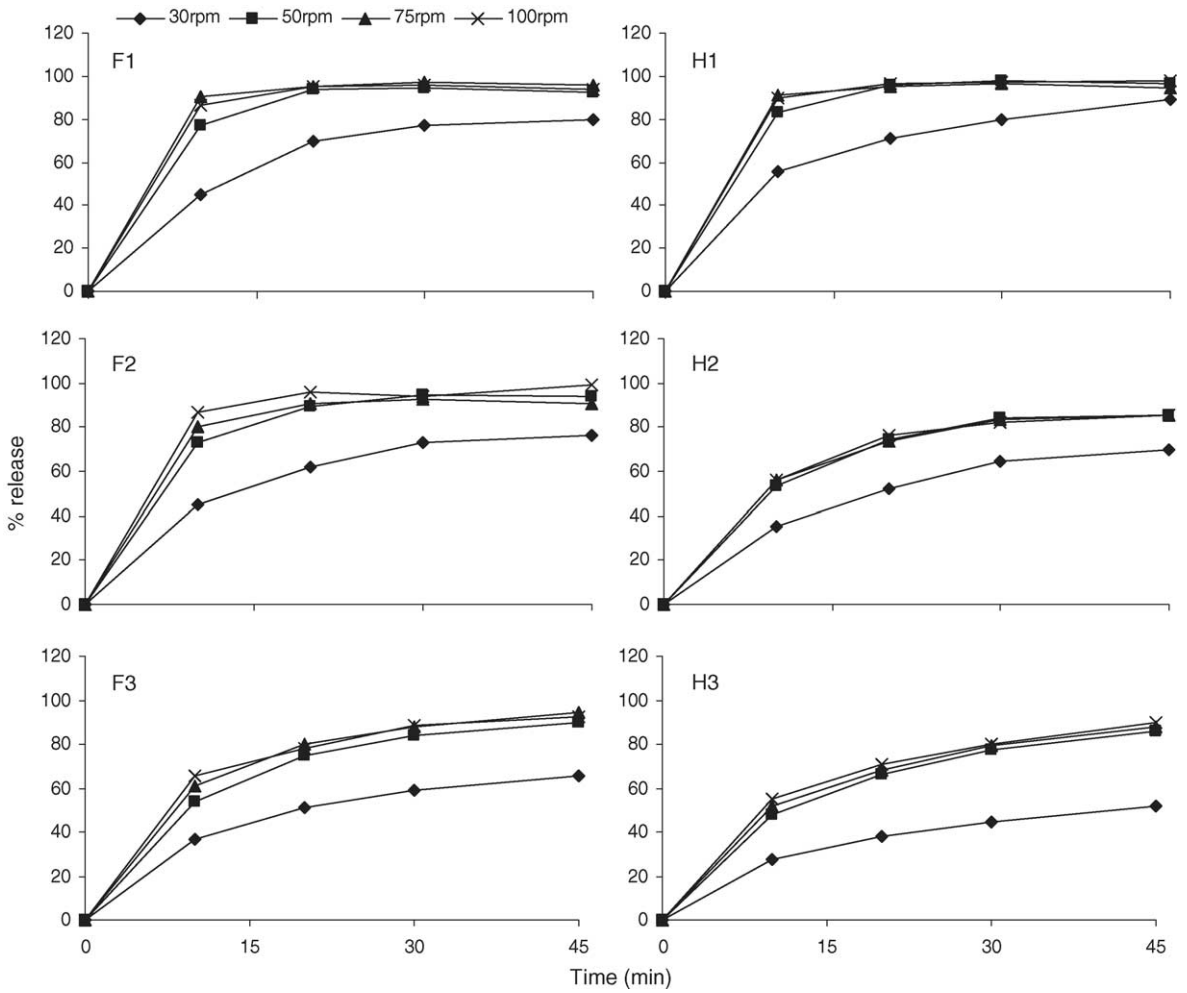

Fig. 3. Dissolution profiles of rifampicin from FDC F and FDC H tablets as a function of agitation intensity and pH of the dissolution medium (F and H represent FDC F and H whereas suffix 1, 2 and 3 represent dissolution in 0.1N HCl, 0.01N HCl and 6.8 pH phosphate buffer, respectively). Dissolution profiles are represented as mean of 5 tablets, SD in first sampling point was less than 10% and for subsequent samples it was less than 5%. These two formulations have shown excellent release profiles with pH and agitation independent dissolution. FDC F and H have passed the bioequivalence test.

1992; Rohrs, 2001). Thus, agitation intensity of 50 rpm was found to be optimum for the discrimination of release of rifampicin from FDC formulations and dissolution profiles at this rotational speed was used for further interpretation.

3.2.2. *Effect of pH*

The important parameter that profoundly influences the solubility and dissolution rate of an ionic drug is pH of GI tract segment (values of the gastric pH in the fasted state fluctuate between 1 and 3 and that of intestinal pH ranges from 5.0 to 7.4 gradually rising between duodenum and ileum) (Dressman et al., 1990, 1998). It is evident from Table 1; rifampicin shows wide variations in the solubility at the physiologic pH. Hence, dissolution of rifampicin was done at 0.1N HCl and 0.01N HCl corresponding to gastric pH and pH 6.8 buffer was selected to simulate intestinal pH. Further, effect of pH on the dissolution of rifampicin from FDCs at 50 rpm is shown in Fig. 4. As seen from Fig. 4,

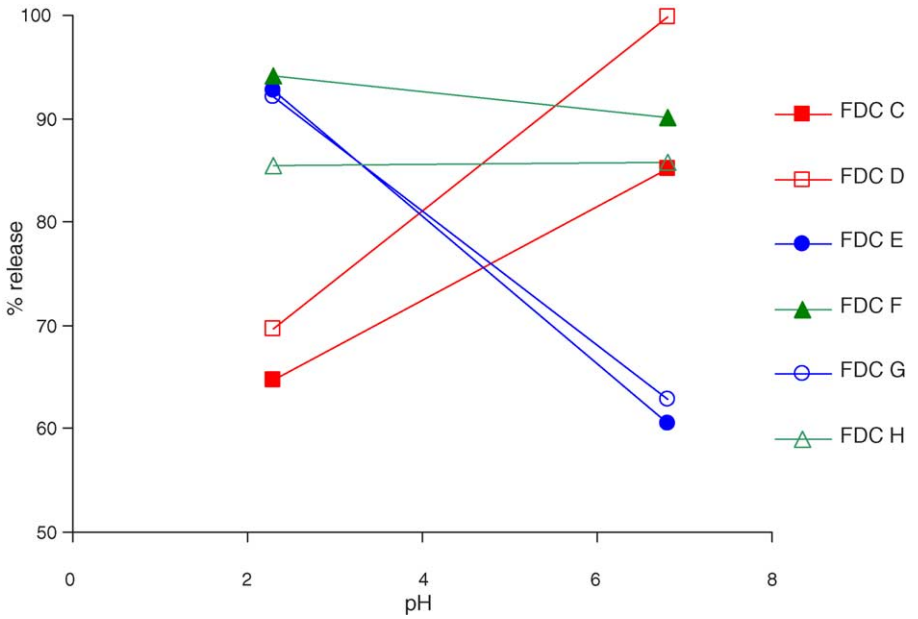

Fig. 4. Dissolution of rifampicin when compared at 0.01N HCl and 6.8 pH buffer. There was no uniform pattern for dissolution of rifampicin compared at 0.01N HCl and 6.8 pH buffer. Formulations FDC F and H have shown pH independent release; dissolution of FDC E and G was retarded at 6.8 pH buffer whereas FDC C and H have shown enhanced dissolution in 6.8 pH buffer. This anomalous behaviour was attributed to the ionic interaction of rifampicin and excipients.

no uniform trend in the dissolution of rifampicin was seen when pH of the dissolution medium was changed from gastric (0.01N HCl, pH 2) to that of intestine (6.8 pH phosphate buffer). Compared to 0.01N HCl, decreased release in 6.8 pH buffer was observed in FDC E (93% versus 60%) and FDC G (92% versus 63%). At the same time, contrary to expected results; increased release was observed in FDC C (64% versus 85%) and FDC D (70% versus 100%) whereas pH independent release was observed in FDC F (94% versus 90%) and FDC H (85% versus 86%). It is pertinent to note here that formulations exhibiting similar behaviour as a function of agitation intensity and pH were from same manufacturers and hence it may be expected that manufacturers have used similar prototype formula for these two formulations. Therefore, based on the pronounced effect of pH on the release of rifampicin from FDCs of different manufacturers, the anomalous behaviour of rifampicin release was attributed to the ionic interaction of rifampicin with the excipients (O'connor and Corrigan, 2002).

In FDC C and D, the most likely mechanism of retardation of extent of dissolution in acidic medium is the interaction of acidic excipients such as sodium lauryl sulfate, docusate sodium with rifampicin resulting in insoluble complex at the acidic pH. Thus, remains as insoluble granules in acidic medium as observed during dissolution even at higher agitation intensity. However, the same complex dissociates rapidly at intestinal pH and thereby increasing not only the rate but also extent of rifampicin dissolution (FDC C and D at pH 6.8). This effect was more pronounced in FDC C that released only 64% in 0.01N compared to 70% release shown by FDC D. In addition, FDC C has shown bigger particles those were settling at the bottom of the dissolution vessel and remained un-dissolved irrespective of higher agitation intensity indicating a stronger interaction with the anionic excipient. In case of FDC D, fine granules were observed after disintegration and hence the total % release was increased when compared to FDC C. The above mentioned results suggest combined effect of type of excipient responsible for ionic interaction and amount or type of disintegrant added on both rate and extent of rifampicin release from FDC formulations. It was reported that sodium lauryl sulfate used as wetting agent to improve dissolution of ri-

Table 6
In vitro–in vivo relationship of rifampicin containing FDC formulations

FDC	C_{max} (μg/ml)	AUC_{0-8} (μg.h/ml)	BE result	Percentage release at 45 min, 50 rpm		
				0.01N HCl	0.1N HCl	6.8 pH buffer
FDC C	3.40	18.00	Failed (↓)	64.67	85.41	85.16
FDC D	7.52	39.81	Failed (↑)	69.70	89.11	99.85
FDC E	5.54	27.31	Passed	92.78	91.40	60.46
FDC F	7.09	31.77	Passed	94.18	92.80	90.12
FDC G	10.16	51.29	Passed	92.16	95.09	62.83
FDC H	9.55	48.10	Passed	85.40	96.54	85.69

Downward arrow indicates that FDC formulation was below the excepted limits of bioequivalence while upward arrow indicates above the limits of bioequivalence. No direct in vitro–in vivo relationship could be obtained since dissolution was dependent on the formulation characteristics that resulted in decreased dissolution in 0.1N HCl where solubility of the rifampicin is maximum. Dissolution at different media corresponding to the physiologic pH has helped in understanding the in vivo behaviour of FDC formulations and its subsequent bioavailability. FDC, fixed dose combination; BE, bioequivalence.

fampicin had negative effect on the in vitro release and bioavailability of rifampicin (Sen et al., 2002). Thus, dissolution study at different pH has provided evidence that formulation parameters such as excipients play an important role in release of rifampicin from FDC formulations, changing rate of in vivo dissolution and thus affecting bioavailability.

3.3. *In vitro–in vivo relationship*

Table 6 gives percentage release of rifampicin at 45 min in different dissolution media at 50 rpm along with C_{max}, AUC_{0-8} and bioequivalence status of these formulations. No direct quantitative relationship was observed between bioequivalence and in vitro release at different agitation or pH due to marked influence of excipients on the dissolution of rifampicin. FDC D that showed comparatively less release in 0.1N HCl (70%) was found to be suprabioavailable. On the other hand, FDC E and G has released only 60% at the pH of absorptive region but still resulted in good bioavailability. Absorption of drugs from GI tract is a very complex process and is influenced by physicochemical, physiologic and formulation factors. Thus, by applying the physiochemical and biopharmaceutics properties of rifampicin to theory behind 'Advanced Compartmental Absorption and Transit (ACAT)' model, it is possible to predict the rate and extent of rifampicin release in relation to its in vitro rate and extent of release at the physiologic conditions (Agoram et al., 2001).

FDC C has shown rapid and complete release in 0.1N HCl, whereas release was retarded in 0.01N HCl corresponding to mean gastric pH of 2. It was also observed that this formulation contained large fraction of the bigger particles in 0.01N HCl. Moreover, with the increase in granule size and density, gastric emptying time also increases, consequently decreasing the amount of rifampicin going to the proximal intestine for absorption (Tuleu et al., 1999; Waterman and Sutton, 2003). Even though FDC C has shown good release at 6.8 pH buffer, this effect was not pronounced in vivo because of the increased gastric residence time of the bigger granules. This reduced concentration at the absorptive site decreases rate of permeation as rifampicin undergoes significant *p*-gp efflux at lower concentration (Agrawal and Panchagnula, 2004a). Thus, the overall amount permeated across the mucosa was reduced because of the decreased dissolution at 0.01N HCl and hence resulted in failure of the bioequivalence test.

On the other hand, FDC D despite of its similar behaviour compared to FDC C, has shown relatively increased release (70% of labeled amount) in 0.01N HCl with smaller particle size of the un-dissolved portion. Further, dissolution of FDC D has increased substantially at pH 6.8. In fact this is the only formulation that released 100% rifampicin in intestinal buffer (Fig. 5 and Table 6). During the in vivo passage of FDC D, 70% rifampicin was released immediately (as evident from Fig. 1) and gastric emptying of granules was not affected, as the particle size was low. These fine granules when reached to intestine resulted in rapid dissolution providing the saturated concentration at the absorptive region. As mentioned earlier, rate of absorption of rifampicin is enhanced at higher concentration

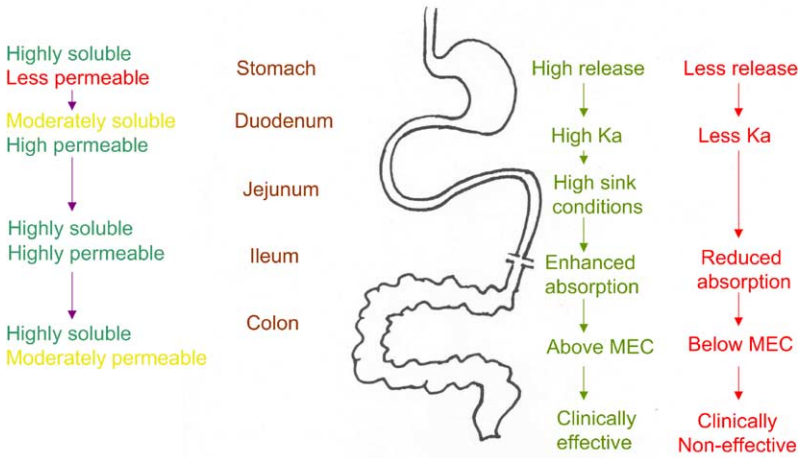

Fig. 5. Schematic representation of influence of dissolution and physiologic factors on the behaviour of rifampicin from FDC formulations Based on the solubility and permeability of rifampicin in the physiologic conditions of GI segments, it can be considered as borderline class II drug. Predicted behaviour of rifampicin containing FDC formulations having different rate of in vitro dissolution is as follows: when a formulation exhibit excellent release profile in 0.01N HCl, the increased concentration at the site of absorption (proximal intestine), the rate of absorption is increased as rifampicin showed concentration permeability values because of the *p*-glycoprotein efflux. Because of high permeability of rifampicin at increased concentration, further sink conditions are provided and formulation result in the appreciable blood levels. However, if a formulation with retarded release is provided to the volunteers, rate of absorption in vivo is reduced because of the less permeability values of the rifampicin at lower concentration resulting in lower plasma profiles and ultimately failure of bioequivalence test.

and thereby resulting in increased amount of permeation from intestine. This predicted sequence of in vivo behaviour of FDC D might have resulted in its supra-bioavailability.

Formulations E and G have shown more than 90% release in 0.01N HCl. In this case with normal gastric emptying time of solutions, rifampicin at the increased concentration was transferred to proximal intestine and thus had increased rate of absorption that resulted in appreciable bioavailability of rifampicin from these two formulations. Although FDC E and G have released only 60% of the labeled amount at intestinal pH buffer, it had no influence on the bioavailability of rifampicin as drug was already in solution form at the preceding GI compartment.

Remaining two formulations FDC F and H have exhibited agitation speed and pH independent release (not less than 85%) in all the three dissolution media (Figs. 3 and 5). Rapid and complete dissolution is desirable for class II drugs in order to maximize the contact between drug and absorbing mucosa. Due to highly permeable nature of rifampicin, it is rapidly removed from GI tract giving less time for degradation, decomposition or movement of drug to the distal parts of intestine. Further, high concentration resulting from rapid release increases rate of rifampicin absorption leading to appreciable bioavailability.

In a nutshell, rifampicin bioavailability from the FDC formulation is an amalgam of dosage form characteristics, physiochemical and physiologic conditions that include rate and extent of rifampicin release as a function of physiologic pH, site and concentration dependent absorption/exorption of rifampicin. This complex and intricate relationship of rifampicin bioavailability from rifampicin containing dosage forms exhibiting different rate of release is illustrated in Fig. 5. While 0.1N HCl does not distinguish any formulation changes due to high solubility, 0.01N HCl having pH 2 provides a better representation of acidity in fasted subjects and can predict the quality of formulation.

Solubility of rifampicin in stomach becomes important because rifampicin is less soluble in the proximal intestine. Importance of gastric solubility in bioavailability of rifampicin is also apparent from the earlier studies where bioavailability of rifampicin is increased after acidification of gastric juice (Keberle, 1970). In another example, when gastric pH was lowered by subcutaneous injection of histamine, high serum levels of rifampicin were obtained, whereas it reduced to half after raising the gastric pH by oral administration of sodium bicarbonate or other antacids (Kenny and Strates, 1981; Khalil et al., 1984). In addition, rifampicin bioavailability was more from syrup/suspension formulations compared to tablets or capsules (Mannisto, 1976). This indicated that rifampicin which is borderline class II drug (Agrawal and Panchagnula, 2004a) exhibits solubility-limited absorption.

This investigation has proved that dissolution at various pH gives better understanding of in vivo behaviour of rifampicin containing formulation and is a valuable clue for the formulation scientist to select appropriate excipient, process or manufacturing conditions and thereby helps in selecting a formulation with most suitable and reproducible release profiles. At the same time, it also explains the earlier disparity of dissolution testing and bioavailability where formulations with good dissolution properties appeared to be poorly absorbed and vice versa. Thus, dissolution methodology has potential to serve as a surrogate to knock out the bad quality formulations in the product development stage itself.

In addition to predicting in vivo performance, classic use of pharmacopeial dissolution test is the quality assurance of a drug product that include ability to confirm batch to batch reproducibility, meeting the specifications according to regulatory requirement and ensuring adequate performance throughout its shelf-life. As can be seen from Figs. 1–3 and Table 5, all the six FDCs have shown rapid and complete release in 0.1N HCl due to its very high solubility in this medium. As variable bioavailability is reported from all types of rifampicin containing dosage forms, estimating the in vitro quality in 0.1N HCl may not be discriminatory in nature and is likely to mask any batch-to-batch variation caused by manufacturing and/or process variables. In addition, all the formulations have met the compendial requirements when dissolution was carried out according to conditions specified for four drug FDC. In this case, high agitation intensity provided by paddle apparatus at 100 rpm might be responsible for indifferent profiles at these conditions. Thus, present pharmacopeial methods do not serve as a discriminatory method for quality evaluations of FDCs. As explained earlier a crucial excipient will considerably change the performance of rifampicin formulations. it is reported in literature that even a change in sequence in addition of components or minor change in manufacturing process had resulted in drastic decrease in rifampicin bioavailability (Fox, 1990). Hence, a readressal of dissolution test conditions for rifampicin containing dosage forms by regulatory agencies is required taking into the consideration the sensitiveness of rifampicin bioavailability to minor changes and its consequences in the treatment of TB. In this regard, 0.01N HCl was found to be a sensitive medium compared to 0.1N HCl and 6.8 pH buffer. The formulation that showed reduced dissolution in 0.01N HCl at 50 rpm has failed the bioequivalence test. As performance of rifampicin is dissolution limited in gastric pH, pharmacopeial dissolution specifications should be reset as 0.01N HCl at 50 rpm using USP type II apparatus.

4. Conclusions

This was the first comprehensive study to correlate in vivo bioavailability and dissolution of FDC formulations at different agitation intensity and pH that has provided an explanation for earlier disparity between in vitro dissolution and in vivo bioavailability. It was found that rifampicin bioavailability is an amalgam of dosage form characteristics coupled with physicochemical parameters in relation to GI tract segment and concentration dependent absorption of rifampicin. In addition, simplified in vitro dissolution methodology can be used as for quality evaluation of rifampicin containing FDC formulations. Based on this comprehensive evaluation, a decision tree is proposed (Fig. 6) which will act as a guideline for quality evaluation of FDC products. It is not only helpful in prior prediction of rifampicin bioavailability from FDC product but also provides a valuable clue to optimize products. In addition, it has a potential to knock out bad quality formulations at the product development stage itself. With the use of this decision tree, chances of false positives are rare, whereas there might be some false negatives

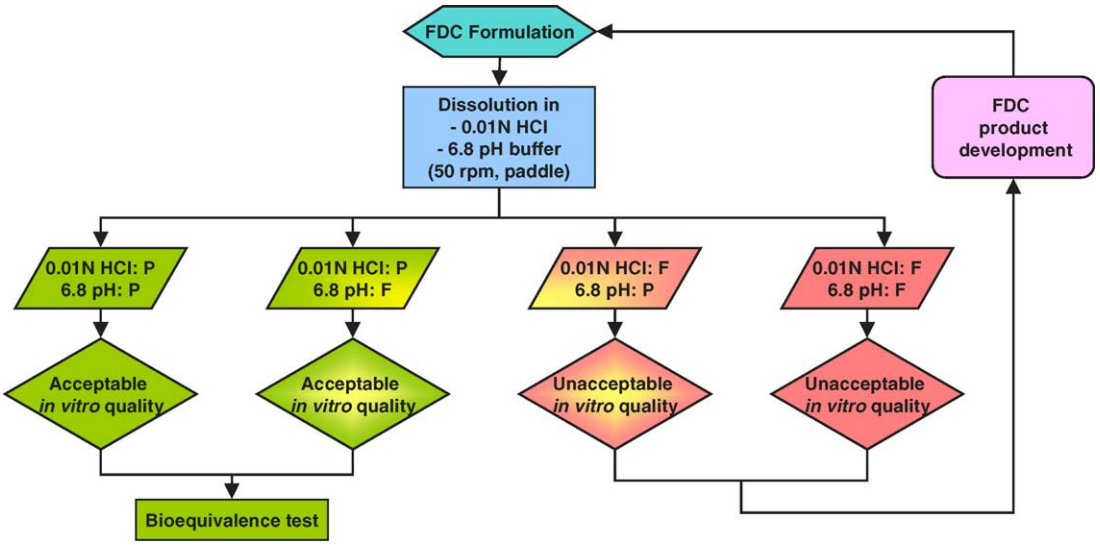

Fig. 6. Dissolution as a surrogate marker for bioequivalence test of rifampicin containing FDC formulations. Dissolution specifications: not less than 75% in 45 min; P: passed, F: failed. Although release in 0.01N HCl is important in judging the acceptable bioavailability of rifampicin, dissolution at 6.8 pH buffer helps in predicting in vivo performance of formulation and provides important clues for FDC product optimization. Formulations with high release (rate and extent) in both the media result in good bioavailability. Formulations showing poor release in 0.01N HCl are likely to show bioavailability problem. Prediction of bioinequivalency from dissolution data early in the product development helps in reducing costly bioequivalence trial requirements. The chances of false positives on the left half of the figure are rare whereas there might be false negatives on the right side because of the complexity of rifampicin absorption based on physiochemical, physiologic factors and concentration at absorption site. However, in both the cases patient safety is ensured. This decision tree has an enormous potential in evaluating formulations to be used in DOTS and marketed products with suspicious bioavailability.

due to sensitiveness of rifampicin bioavailability on physicochemical and physiologic factors. In both the cases, TB patient is benefited in a respect that good quality FDC reaches to the patient. More importantly, this methodology is helpful in evaluating all the rifampicin containing solid oral formulations in the market having uncertain/undetermined bioavailability which otherwise is a formidable task. Additionally, formulations to be used in directly observed treatment-shortcourse (DOTS) for the resource poor countries can be evaluated using this simplified methodology for the assurance of rifampicin bioavailability and treatment of TB with good quality FDC formulations.

Acknowledgements

Authors are thankful to Kanwaljit Kaur, Inderjit Singh, and Shantaram Badhe for their skillful technical input during the project. Financial support from TDR, WHO and encouragement of Dr. Tom Kanyok, TDR is appreciated and put on record.

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