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In vitro evaluation of fixed dose combination tablets of anti-tuberculosis drugs after real time storage at ambient conditions

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Rifampicin exhibits variable bioavailability from solid oral dosage forms and this problem is more apparent when it is formulated as fixed dose combination (FDC) in presence of other first-line anti-tuberculosis drugs. To determine the cause of variable bioavailability, the aging effect on physical and chemical performance of rifampicin from FDC formulations after real time storage at the ambient conditions was investigated. For this purpose, six FDC formulations from different manufacturers were stored at ambient conditions (20–35 °C, with no control of humidity) in the final packing for a period of 16–38 months and its *in vitro* quality control tests for rifampicin were compared with the initial performance of these tablets. None of the formulations have shown significant weight gain/loss and the assay values were within the pharmacopeial limits when evaluated by a stability indicating method. Further storage had no effect on physical performance of FDC tablets as indicated by unaltered dissolution profiles. Formulation reevaluation after real time storage at the ambient conditions for 16–38 months indicated that rifampicin containing FDC formulations are stable throughout its shelf life and instability is not a cause of variable bioavailability.

1. Introduction

Rifampicin is an important component of tuberculosis (TB) therapy, which is known to exhibit variable bioavailability especially from fixed dose combinations (FDC) with other first line anti-TB drugs such as isoniazid, pyrazinamide and ethambutol. To understand the causes of variable bioavailability of rifampicin from FDC formulations, we have undertaken a systematic and comprehensive evaluation of physico-chemical, physiologic and formulations factors and subsequently its retrospective correlation with the bioavailability of rifampicin from FDCs. Among these factors, storage may have deleterious effects on the product performance. When evaluating the stability of a formulation, both physical and chemical properties must be considered thoroughly. In solid state, chemical reactions are very slow but may be accelerated by temperature, light, pH, humidity, radiation and pressure. These reactions are to be considered in evaluating the stability of tablet formulation, predicting shelf life and creating optimum storage conditions. According to International Conference on Harmonization (ICH) guideline, the stability of the formulation is predicted from accelerated conditions of humidity and temperature (40 °C/75% RH for 6 months) in the final packaging or particular container closure system for the marketing and based on stability data shelf life is determined (ICH 2000). However, in some cases accelerated stability is not always indicative of long term stability of the product because of the different physico-chemical properties of the pharmaceutical substances and/or excipients at elevated conditions (Fitzpatrick et al. 2002). Hence, if a significant change is observed in accelerated testing any extrapolations of stability data need to be confirmed by studies at intermediate storage conditions (30 °C/60% RH) or long term stability studies (20 °C/60% RH) for zone I and II whereas for zone III and IV the storage conditions mentioned are 30 °C/65% RH (ICH 2002 a and b; WHO 2001). In this regard, the shelf life of rifampicin containing fixed dose combination (FDC) products is 24–30 months when stored in the airtight containers/packing and protected from light (as mentioned on the product labels of the FDC formulations).

Apart from the chemical stability of the active ingredients in the pharmaceutical dosage forms under extended shelf storage, physical changes in the formulation affecting the dosage form characteristics could be equally deleterious (Saville 2001). Examples of physical changes that can take place in solid oral dosage forms and may affect the therapeutic effectiveness of the formulation are change in crystal form, increased or decreased disintegration and dissolution times etc (Desai et al. 1994). It is reported that aging decreased the dissolution efficiency of super-disintegrants such as croscarmellose and crospovidone in wet granulated tablets and thus can influence bioavailability (Gordon et al. 1993). Hence, evaluation of physical performance along with the chemical stability of the formulation at the end of the shelf life is important to check for the possible effects of physical

changes on the bioavailability by carrying out dissolution studies.

In light of the above discussion, FDC formulations used in the eight bioequivalence studies at NIPER bioavailability center were evaluated for physical and chemical performance of rifampicin after 18–36 months of storage at the ambient conditions covering the complete shelf life to determine the extent of deterioration (if any) of FDC formulations during its shelf life.

2. Investigations, results and discussion

Except samples G and H, all FDC formulations were expired at the time of reevaluation, whereas these two formulations were stored for more than 16–17 months. All other separate formulations were at the various stages of the shelf life depending on the drug (2–5 years). None of the formulations showed significant weight gain, discoloration or visual signs of moisture uptake during the storage at uncontrolled humidity conditions throughout its shelf life and after the expiration period. In general sugar coated tablets and capsules are more sensitive to moisture uptake (Saville 2001), however, rifampicin formulations present in all the forms such as capsules, sugar coated and film coated tablets did not show any visual changes. Further, tablets containing ethambutol, which is a known hygroscopic drug did not show any sign of moisture uptake at differing humidity conditions.

Physical and chemical performance of six FDC (C–H) formulations after long term storage compared to initial performance at the time of the bioequivalence study is summarized in the Table. None of the FDC tablets (three drug or four drug combinations) showed significant weight gain. Further, the chemical stability of rifampicin from FDC tablets was checked by a stability indicating assay and it was found that all the formulations passed the pharmacopeial test (90–110%) even after storage beyond their shelf life (Fig. 1). Performance of FDCs in relation to bioavailability after storage was evaluated by dissolution studies. Dissolution profiles of these formulations are shown in Fig. 2 and extent of release and f_2 values are given in the Table. As indicated by a similar release behaviour and f_2 values grater than 70, the performance of all the FDC tablets is unaltered by a long storage period. Absence of any difference in the dissolution rate of FDC formulations after 2–5 years of storage indirectly proved that there is no adverse effect of any excipient during storage. Thus, FDC formulations were found to be physically as well as chemically stable throughout their shelf life when present in the final packing and stored at ambient conditions.

It is pertinent to mention here that all the six FDC formulations have shown varied *in vivo* bioavailability of rifampicin. Out of these six formulations, FDC C was below the bioequivalence limits for C_{max}, whereas FDC D had shown increased bioavailability both in terms of AUC and C_{max}. Unaltered *in vitro* performance of rifampicin from all these formulations even after long term storage beyond the shelf life irrespective of its *in vivo* variability and type of FDC indicated that storage of the formulations has no effect on the rifampicin bioavailability. Thus, absence of any significant change in weight, assay and dissolution properties of rifampicin from six FDC tablets of different manufacturers after real time storage at ambient conditions proved that FDC formulations show no stability problems when stored under appropriate conditions.

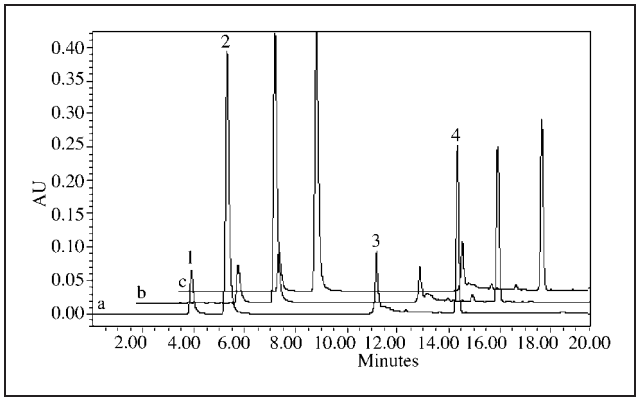

Fig. 1: Representative chromatograms of assay of FDC tablets done after 18–36 months of storage at ambient conditions (a: Standard drugs, b: FDC D and c: FDC H). Numbered peaks in the chromatogram represent
1: isoniazid, 2: pyrazinamide, 3: peak due to change in mobile phase composition during gradient run and 4: rifampicin peak.
After the long storage, formulations have passed the pharmacopeial assay test for rifampicin, isoniazid and pyrazinamide as assay values for all the six FDC tablets were well within the range of 90–110% (USP 26, 2003)

Table: Physical and chemical performance of FDC tablets evaluated for rifampicin after real time storage throughout its shelf life and analyzed after 16–38 months of storage

Formulation	BE study	Storage (months)	Weight (g)			Assay (%)			Dissolution (% release in 45 min)		
			BE study	Aug 2003	% difference[1]	BE study	Aug 2003	Assay test[2]	BE study	Mar 2003	f_2[3]
FDC C	Jan 2000	38	1.2202	1.2181	−0.17	101.87	94.87	Passed	86.17	84.39	75.88
FDC D	Apr 2000	35	1.2518	1.2510	−0.06	105.55	98.64	Passed	85.00	90.11	78.31
FDC E	Jul 2000	32	1.7102	1.6979	−0.72	101.24	95.20	Passed	100.00	98.83	92.84
FDC F	Jul 2000	32	1.2539	1.2518	−0.17	100.80	95.58	Passed	102.41	97.68	73.00
FDC G	Oct 2001	17	1.0593	1.0600	0.07	98.89	106.96	Passed	109.91	104.01	75.51
FDC H	Nov 2001	16	0.8532	0.8555	0.27	102.65	98.00	Passed	103.25	95.31	71.11

[1] There was no significant weight loss or gain in any of the six FDC formulations.
[2] Pharmacopeial assay limit of rifampicin from FDC formulations: 90–100% (USP, 2003).
[3] f_2 values more than 50 indicate the unchanged dissolution profiles of rifampicin from FDC tablets performed after 16–36 months storage. Formulations A and B were not available in sufficient quantity for the reevaluation.
FDCs C–F were expired at the time of reevaluation for rifampicin.
FDCs C–G were four drug FDC tablets containing rifampicin, isoniazid, pyrazinamide and ethambutol whereas FDC H was three drug FDC containing rifampicin, isoniazid and pyrazinamide.
Sample size for weight variation, dissolution and assay was 20, 5 and 3 respectively and the resultant coefficient of variation was always less than 1% for weight variation, 5% for dissolution and 3% for assay.
Irrespective of the variable bioavailability of these formulations, its *in vitro* performance remained unchanged even after storage beyond the shelf life.
Abbreviations: BE: bioequivalence, f_2: similarity factor.

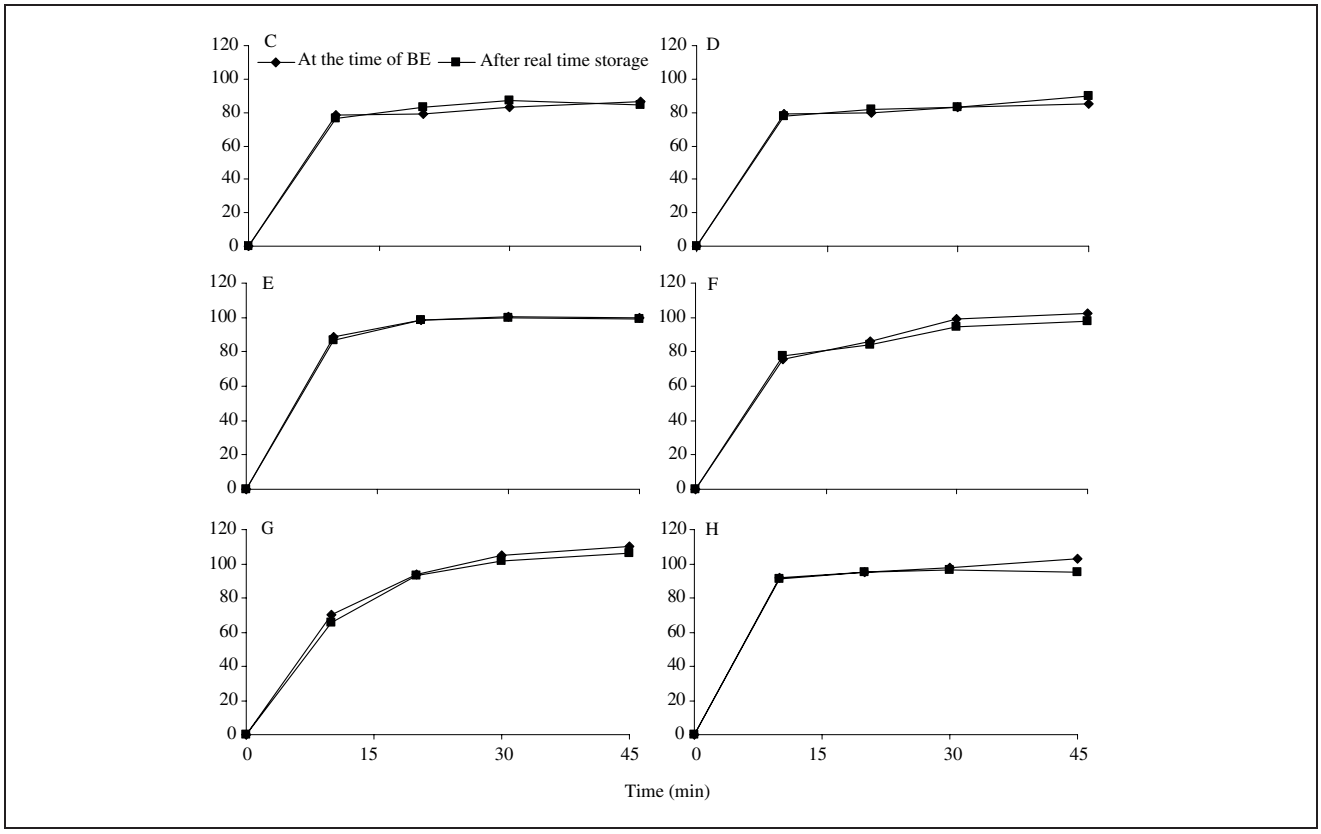

Fig. 2: Dissolution profiles of FDC tablets at the time of conduction of bioequivalence study and after real time storage of 18–36 months.
Percentage release at each time point before and after storage was not statistically different at 95% confidence interval.
Coefficient of variation was always less than 10% for first sampling point that reduced to less than 5% for subsequent samples.
Rifampicin dissolution profiles before and after the real time storage were not significantly different from each other as indicated by f_2 values greater than 70.

3. Experimental

3.1. 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). Symmetry C_{18} precolumn and column were procured from Waters Associates (Milford, MA, USA). Freshly prepared de-ionized water was used in the all the dissolution studies whereas ultra-pure water prepared by reverse osmosis and filtered through 0.45 μm membrane filter was used for HPLC analysis.

3.2. Instruments

Waters HPLC system (Milford, MA, USA) consisting of multisolvent delivery pump 600 E, 717 plus autosampler and 2487 dual λ absorbance detector with Millennium^{32} software (version 3.20) was used for analysis of assay samples. All the dissolution studies were performed with an Electrolab tablet dissolution tester (USP XXIII) (Mumbai, India) and samples were analyzed on a Beckman DU® 640i spectrophotometer (Fullerton, CA, USA). Other instruments used include a Thermo-Orion digital pH meter attached to a glass electrode (Beverly, MA, USA), Elgastat, (ELGA Ltd. Bucks, UK), an electronic balance AG 245 (Greifensee, Switzerland), a Branson 3210 sonicator (The Hague, The Netherlands), a Millipore syringe filtration assembly (Bangalore, India), Brand autopipettes from E. Merck (Mumbai, India) and microlitre syringes from Hamilton (Bonaduz, Switzerland).

3.3. Storage conditions and methodology of evaluation

All the formulations of the eight bioequivalence studies (A–H) were visually observed for changes in the color or apparent moisture absorption after storage for 21–63 months. All the formulations were stored in ambient room temperature of 20–35 °C with no control of the humidity, in the containers or the packing received from the manufactures (either as bulk dispensing packs in HDPE bottles or as aluminum strips). Humidity conditions generally varied in the range of 10–40% RH in Mar–Jun, 60–99% in Jul–Sep and 50–85% in Oct–Feb depending on the climatic conditions at different periods of the year (data taken from a local news magazine). The evaluation was based on visual changes due to moisture absorption and weight of all the dosage forms (FDCs and separate formulations of rifampicin, isoniazid, pyrazinamide and ethambutol).

3.4. Evaluation of FDC tablets for rifampicin after real time storage at ambient conditions

To evaluate the storage/aging effect on the performance of rifampicin from FDC formulations, six FDC tablets (C–H) were evaluated for assay and dissolution after real time storage under the above-mentioned conditions. For two studies A and B, formulations were not sufficient and hence weight variation assay, and dissolution could not be performed. Out of six FDC formulations studied for aging effect, FDCs C–G were four drug FDCs containing rifampicin, isoniazid, pyrazinamide and ethambutol whereas FDC H was a three drug FDC containing rifampicin, isoniazid and pyrazinamide.

3.4.1. Assay

Initial assay values at the time of conduction of the bioequivalence studies were taken as supplied by the respective manufacturer. After storage for 18–36 months, the assay was done by a modified method described in the USP 26 (USP 2003). For this purpose, an individual tablet was crushed and whole contents was transferred to 250 ml volumetric flask containing 200 ml of methanol and 6.8 pH phosphate buffer (50 : 50). This was then sonicated for 15 min, allowed to equilibrate to room temperature and the volume was made up. The mixture was filtered through Whatman filter paper (No. 1) and after discarding the initial filtrate, 10 ml of this solution was diluted to 100 ml with phosphate buffer. Thus final solution obtained was filtered through a polycarbonate filter (0.4 μm) and analyzed immediately by a stability indicating HPLC method described in USP 26. Assay of the all the formulations was done in triplicates.

3.4.2. Dissolution

Dissolution tests were conducted using a six stage dissolution with USP II (paddle) specifications at 37 ± 0.5 °C using 900 ml of dissolution medium (0.1N HCl). For each dissolution test five tablets were used and a sixth vessel was used for reference in which pure drugs equivalent to the

amount present in the formulation were dissolved (USP 2003). Samples (5 ml) were withdrawn at 10, 20, 30 and 45 min with replacement, diluted with dissolution medium and analyzed immediately by colorimetry at 475 nm, a method which was validated against the HPLC method of analysis (Agrawal et al. 2004). The percentage release of rifampicin was calculated with respect to the reference vessel. Dissolution data is expressed as percentage (%) of labeled amount released in 45 min (D_{45}) and the profiles were evaluated statistically by calculating the similarity factor (f_2 value). According to this method, a f_2 value between 50 and 100 suggests that two dissolution profiles are similar (Polli et al. 1997).

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