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Comparative bioavailability of rifampicin, isoniazid and pyrazinamide from a four drug fixed dose combination with separate formulations at the same dose levels

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Abstract

Fixed dose combination (FDC) formulations became popular in the treatment of tuberculosis (TB) because of the better patient compliance, reduced risk of monotherapy and emergence of drug resistance in contrast to treatment with separate formulations of two to four first-line drugs. However, its successful implementation in national programs is limited by probable bioinequivalency of rifampicin if present in FDC form. In this regard, World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) recommend FDCs only of proven bioavailability. Hence, bioequivalence study of four drug FDC tablet was conducted using 22 healthy male volunteers according to WHO recommended protocol to determine bioavailability of rifampicin, isoniazid and pyrazinamide compared to standard separate combination at the same dose level. The study was designed as two period, two treatment crossover experiment with a washout period of 1 week. Bioequivalence of rifampicin was estimated by plasma and urinary method for both rifampicin and its active metabolite, des-acetyl rifampicin whereas isoniazid and pyrazinamide were estimated from plasma. Mean concentration time profiles and all the pharmacokinetic parameters of rifampicin, isoniazid and pyrazinamide from FDC tablet were comparable to individual formulations and passed the bioequivalence test with power of the test above 95%. Further, bioequivalence of both rifampicin and isoniazid shows that in vitro interaction of rifampicin and isoniazid is clinically insignificant. Thus, it was concluded that FDC formulation is bioequivalent for rifampicin, isoniazid and pyrazinamide and ensures the successful treatment of TB without compromising therapeutic efficacy of any of these components of anti-TB therapy.

Keywords: Bioavailability; Bioequivalence; Fixed dose combination; Isoniazid; Pyrazinamide; Rifampicin; Tuberculosis

1. Introduction

Tuberculosis (TB) is a complex socio-economic disease that apart from its alarming death statistics is a marker of social inequity and serious impediment in the economic development (WHO, 2002). For the effective treatment of TB and to reduce the number of drug resistant strains, World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) encourage use of Fixed Dose Combination (FDC) formulations that evolved from the fact that TB always require multi-drug

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treatment (Blomberg and Fourie, 2003). However, in spite of the numerous advantages offered by FDC formulations over separate formulations in the treatment of TB, only an estimated 24% of notified cases are treated with two and/or three drug FDCs in public sector (Norval et al., 1999). In this regard, inferior quality of FDC formulations is considered as the major limitation in its widespread use since the treatment outcome is questionable (Blomberg et al., 2002; Laing et al., 1999). It is reported that rifampicin which is a major component of anti-TB cocktail, exhibits variable bioavailability when combined in FDC forms. It is pertinent to note that bioavailability of some FDCs when compared with rifampicin alone is not negatively affected (Zwolska et al., 1998) while in some cases comparison with separate formulations at same dose levels have shown reduced bioavailability (Acocella, 1989; Padgoankar et al., 1999). Interestingly, some of the trials report increased relative bioavailability of rifampicin from FDC formulations (Nyazema et al., 1999). Although dissolution testing is considered as an important tool for quality evaluation of the formulations, this holds poor in relation to rifampicin bioavailability from FDCs. While FDC formulations with good dissolution were poorly absorbed (Acocella, 1989), formulation with a poor dissolution had exhibited good bioavailability (Aspesi, 1989; Laing et al., 1999). As at present there is no in vitro surrogate test available to monitor the quality of FDC formulations, WHO and IUATLD recommend only those FDCs of proven bioavailability (Anonymous, 1994) and further developed a simplified screening protocol for the assessment of rifampicin bioequivalence that utilizes data of only six blood samples over a period of 8 h from 20 volunteers and is considered as convenient and cost-effective (Fourie et al., 1999). To further simplify the evaluation of quality of FDCs, WHO and IUATLD have identified two reference laboratories with the proven efficiency especially in high prevalence, low-income countries to expedite the process of proving the quality of FDCs and thus facilitating its implementation in the national TB programs. National Institute of Pharmaceutical Education and Research (NIPER) is one of the two centers worldwide with the WHO accreditation to carry out bioequivalence trials of anti-TB formulations and since then has a valuable contribution in the FDC evaluation project of WHO by its active involvement in assessment of FDC quality to be registered for marketing (Panchagnula et al., 1999a,b, 2000; Agrawal et al., 2002a,b).

This investigation was carried out with a purpose to test the bioequivalence of rifampicin from four drug FDC formulation when compared to standard separate formulations at the same dose levels prior to its registration for the marketing approval. Apart from variable bioavailability of rifampicin from FDCs, it is reported in the literature that isoniazid aids in situ degradation of rifampicin in acidic conditions and in this process, isoniazid in presence of rifampicin in 0.1N HCl degrades to 3.52% in 15 min and 10.32% in 3h (Singh et al., 2000). To determine the in vivo influence of this interaction on isoniazid bioavailability and to ensure the therapeutic concentration at the tissue levels, assessment of bioequivalence of isoniazid was also one of the objectives. In addition, with the bioanalytical method for isoniazid developed in our laboratory, pyrazinamide can also be analyzed using same extraction method and analytical conditions with no additional cost (Agrawal et al., 2001). Thus, in this study, the bioequivalence of three components, viz. rifampicin, isoniazid and pyrazinamide was determined from four drug FDC tablet of rifampicin, isoniazid, pyrazinamide and ethambutol when compared to corresponding separate formulations.

2. Materials and methods

2.1. Materials

Rifampicin, des-acetyl rifampicin, rifapentine, isoniazid, pyrazinamide and ethambutol were supplied by Lupin Laboratories Ltd., Mumbai, India. All other reagents were of analytical or chromatographic grade procured from Loba Chemie, Mumbai, India; Sigma Chemical Company, St. Louis, USA and Mallinckrodt, France. Ultra pure water (18.2 MΩ) filtered through 0.45 μm membrane filter (Millipore, USA) was used in all the experiments.

2.2. Instrumentation

Waters HPLC system (Milford, MA, USA) consisting of two 515 pumps, 717 plus autosampler and 2487 dual λ UV-Vis detector was used. Millennium software (version 2.1) was used for data acquisition and

processing. Other instruments used include electronic balance AG 245 (Greifensee, Switzerland), Branson 3210 sonicator (The Hague, The Netherlands), Beckman DU® 7600 spectrophotometer (Fullerton, CA, USA), Electrolab tablet dissolution tester (USP XXIII) (Mumbai, India), Thermo-orion digital pH meter attached to glass electrode (Beverly, MA, USA), Maxi dry lyo from Heto Holten (Allerod, Denmark), Biofuge primo from Heraeus (Hanau, Germany), Brand autopipettes from E Merck (Mumbai, India) and microlitre syringes from Hamilton (Bonaduz, Switzerland) and Elgastat (ELGA Ltd. Bucks, UK).

2.3. Formulations

FDC formulation utilized in this study was film-coated four drug FDC tablet consisting of 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide and 275 mg ethambutol (Forecox Trac, Macleods Pharmaceuticals Ltd, Mumbai, India). All the drugs present in this formulation were according to WHO recommended dose ratios and the strength was according to four drug FDC tablet incorporated in WHO list of essential medicines (Blomberg et al., 2002). As the study was conducted using 600 mg dose of rifampicin, four FDC tablets were given to each volunteer according to the administration sequence. Separate formulations used were rifampicin capsules (2 × 300 mg; Rimactane 300, Novartis India Pvt Ltd, Pune, India), isoniazid tablet (1 × 300 mg; Isonex Forte, Pfizer Ltd, Mumbai, India), pyrazinamide tablets (2 × 800 mg, Macleods Pharmaceuticals Ltd), and ethambutol tablets (2 × 550 mg, Macleods Pharmaceuticals Ltd). Before conducting the bioequivalence study, formulations were subjected to pharmaceutical quality control with respect to uniformity of tablet weight, content of active drug substance and dissolution according to pharmacopeial conditions (Supplement 2 to USP 24, 2000).

2.4. Conduction of bioequivalence trial

2.4.1. Experimental design

The study was conducted following WHO protocol (Fourie et al., 1999). The clinical trial was designed as single-dose, two-treatment, two-period crossover with 1 week washout period utilizing 22 (considering two dropouts) healthy male volunteers after getting approval from the NIPER Ethical Committee. The NIPER Ethical Committee is equivalent to Institutional Review Board (IRB) of western developed countries which is an independent body duly constituted with both NIPER faculty and outside specialists from the field of medicine and pharmacy.

2.4.2. Inclusion criteria

A group of people was screened by performing physical examination, liver function tests, hemogram, HBV, routine urine analysis and chest X-ray. After screening, 22 healthy subjects aged between 18 and 55 years and weighing more than 50 kg with no history of drugs or alcohol abuse, liver kidney or gastro-intestinal disorders were selected. The scope of the study was explained to them and each one signed an informed consent form before onset of the study.

2.4.3. Dosing schedule

On each experimental session, after overnight fast formulations were swallowed on an empty stomach with a glass of water (approximately 200 ml). A light breakfast and lunch was provided after 2 and 6 h, respectively. A group of 22 healthy subjects received either four FDC tablets (the test formulation containing 150 mg of rifampicin, 75 mg of isoniazid, 400 mg of pyrazinamide and 275 mg of ethambutol) or standard separate drug formulations (two rifampicin tablets containing 300 mg rifampicin each, one isoniazid tablet having 300 mg of isoniazid, two pyrazinamide tablets having 800 mg of pyrazinamide each and two ethambutol tablets having 550 mg of ethambutol each). The administration sequence was randomized in order to reduce the sequence and period effect. The record sheets of blood and urine specimens' collection and a symptoms checklist with details of adverse reactions of the clinical study were prepared on both occasions for each subject.

2.4.4. Blood sampling

Venous blood samples (3 ml) were collected into heparinized vacutainers using indwelling catheters at 0 h (during the implantation of catheter just before the administration of dose), 1, 2, 3, 4, 6, 8, 12 and 24 h after ingestion of the dose at each experimental session. The samples were immediately centrifuged at 8000 rpm for 20 min. The plasma was separated into tubes containing ascorbic acid (0.5 mg/ml of plasma)

to prevent oxidative degradation of rifampicin and stored at −20 °C till analysis.

2.4.5. Urine sampling

All the subjects were asked to empty their bladder before the ingestion of dosage. This urine sample was discarded. All the urine collections were pooled from 0 to 4 h (they were asked to empty their bladder immediately after giving their 4 h blood sample) and 4–8 h (all the subjects emptied their bladders immediately after 8 h blood sample). After thoroughly mixing each urine collection pH and volume were recorded. Ascorbic acid (0.5 mg/ml of urine) was added to an aliquot (20 ml) and rest of the collected urine was discarded. After proper labeling these urine samples were stored at −20 °C till analysis.

2.5. Bioanalytical work

Plasma samples of both the periods of one volunteer were processed in one day along with six calibration standards and three quality control samples. While in case of urine, all the samples were analyzed in 4 days. Either a calibration curve standard or quality control sample was injected after every two samples of volunteer plasma or urine.

2.5.1. Analysis of rifampicin and des-acetyl rifampicin

Rifampicin and its active metabolite desacetyl rifampicin from plasma as well as urine were analyzed by a validated high performance liquid chromatography (HPLC) method (Panchagnula et al., 1999c). Rifapentine was used as an internal standard and separation was achieved on Nova Pak C_{18} (250 mm × 4.6 mm i.d., 4 μm) column. Mobile phase omposition was methanol: sodium phosphate buffer (pH 5.2; 0.01 M) (65:35, v/v) and detection was done at 254 nm. At a flow rate of 1 ml/min peaks of parent drug, its metabolite and internal standard were well resolved without the interference of isoniazid, pyrazinamide and their metabolites within run time of 20 min.

2.5.2. Analysis of isoniazid and pyrazinamide

Analysis of isoniazid and pyrazinamide from plasma samples was done by a method developed in this laboratory. Analytical column used was reversed phase Spherisorb C_8 (250 mm × 4.6 mm i.d., 4 μm) with mobile phase composition of methanol, water, perchloric acid (70%) and tetra butyl ammonium hydroxide (40%) (2:8:0.005:0.0025). At a flow rate of 1 ml/min and detection at 267 nm, there was no interference of plasma artefacts, metabolites or other drugs in the analyte peaks. Analysis of isoniazid in this method is based on the derivatization with *p*-hydroxy-benzaldehyde resulting in the formation of hydrazone, which is more hydrophobic than plasma artefacts. The method is validated for linearity, range, accuracy, precision and system suitability parameters and are described in detail elsewhere (Agrawal et al., 2001).

2.6. Calculation of pharmacokinetic parameters

All the pharamcokinetic parameters were determined by non-compartmental analysis (Ritschel and Kearns, 1999). $AUC_{0–8}$, $AUC_{0–12}$, $AUC_{0–24}$ and $AUC_{0–inf}$ were calculated by linear trapezoidal method. C_{max} (the highest drug level measured) and T_{max} (the time to reach the highest concentration) were directly read from the concentration time plots. Elimination rate constant (K_{el}) was estimated from the linear regression line of the elimination phase. Elimination half-life ($t_{1/2}$) was calculated as $0.693/K_{el}$.

2.7. Statistical analysis

Bioequivalence assessment for $AUC_{0–8}$, $AUC_{0–12}$, $AUC_{0–24}$, $AUC_{0–inf}$ and C_{max} were done by non-parametric Hauschke analysis (Hauschke et al., 1990; Fourie et al., 1999; Panchagnula et al., 1999a) by two one-sided test at 90% confidence interval. In addition, power of the test was also calculated using the maximum variation in absorption shown by the formulations. FDC formulation was considered bioequivalent to separate formulations if it passes the requirement of 0.80–1.25.

3. Results and discussion

In the product development or manufacturing of the FDCs, rifampicin is the only water insoluble component and hence its incorporation with other highly water-soluble drugs is a critical process which is further complicated by number of processing steps like

grinding, mixing, granulation and drying that may alter the crystalline nature, particle size, dosage form characteristics and release behaviour thereby affecting its bioavailability (Agrawal et al., 2004a,b; Laing et al., 1999; Cavenaghi, 1989; Buniva et al., 1983; Sen et al., 2002). In addition, common pharmaceutical excipients used in the tablet, such as binder, glident may adversely affect rifampicin release subsequently reduces its GI absorption (Agrawal and Panchagnula, 2004; Boman et al., 1975). Further, pharmacopeial in vitro dissolution test does not correlate with in vivo release and absorption of rifampicin from GI tract (Agrawal and Panchagnula, 2004). Hence in order to ensure the quality of four drug FDC tablet before reaching the consumers, its bioequivalence was tested compared to standard separate drugs at the same dose levels. All the formulations used in the study contained the active ingredients within the pharmacopeial limits and passed the other in vitro tests such as weight variation and dissolution. This study was conducted according to WHO simplified protocol that suggest use of 20–22 subjects and sampling points upto 8 h. Although it was proved that for rifampicin bioequivalence sampling points upto 8 h ensures both rate and extent of absorption and enables its comparison with the test product (McIlleron et al., 1999; Agrawal et al., 2004a,b), additional sampling points were collected at 12 and 24 h as half life of isoniazid is 5–7 h for slow acetylators and that of pyrazinamide is 8–10 h (Anonymous, 1999) which was also the objective of this investigation due to in vitro interaction of rifampicin and isoniazid in acidic conditions.

Mean plasma concentration time profiles of rifampicin and its active metabolite des-acetyl rifampicin is shown in Fig. 1, whereas all the pharmacokinetic parameters are given in Table 1. As 10% of the rifampicin dose is excreted unchanged in urine and its metabolite also posses the anti-TB activity, bioequivalence of rifampicin was also tested by urinary excretion method over a collection period of 8 h (Kenny and Strates, 1981; Ellard and Fourie, 1999, Fourie et al., 1999) and is shown in Fig. 2. For isoniazid and pyrazinamide, mean concentration time profiles are shown in Figs. 3 and 4 whereas its pharmacokinetic parameters are listed in Table 1. Upper and lower limits of the bioequivalence obtained after statistical evaluation for all the drugs are given in Table 2.

It is evident from Fig. 1, that rifampicin in vivo performance from FDC formulation was similar to that from separate formulation. In both the formulations, between-subject variation in the ratio of combined to separate for AUC and C_{max} was 20–23%, however, it exhibited less within-subject variation than between-subject variation. The pharmacokinetic parameters for both combined and separate formulation as given in Table 1 were comparable

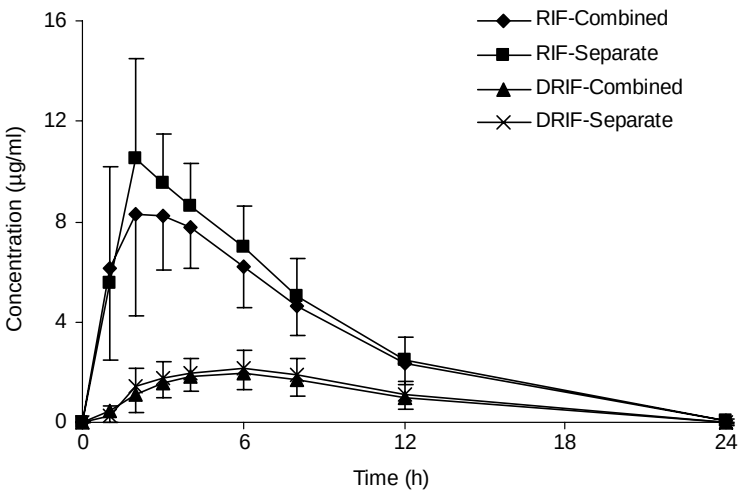

Fig. 1. Concentration time profiles of rifampicin and des-acetyl rifampicin from plasma when given as combined and separate formulations. *Y*-error bars indicate S.D., $n = 22$. Abbreviations: RIF: rifampicin, DRIF: des-acetyl rifampicin.

Table 1
Mean pharmacokinetic parameters of anti-tuberculosis drugs from combined and separate formulations

Pharmacokinetic parameter	Rifampicin		Des-acetyl rifampicin		Isoniazid		Pyrazinamide	
	Combined	Separate	Combined	Separate	Combined	Separate	Combined	Separate
AUC_{0-8} (μg h/ml)	51.3 ± 10.8	57.6 ± 14.0	11.5 ± 3.6	12.6 ± 4.2	22.0 ± 8.1	22.8 ± 7.3	186.7 ± 28.8	186.4 ± 26.5
AUC_{0-24} (μg h/ml)	79.6 ± 17.1	87.6 ± 23.3	23.1 ± 8.2	25.0 ± 9.5	28.5 ± 12.5	30.4 ± 13.1	361.5 ± 77.0	362.1 ± 71.7
AUC_{0-inf} (μg h/ml)	80.1 ± 16.9	87.9 ± 23.7	23.5 ± 8.7	25.0 ± 9.5	19.2 ± 13.3	31.2 ± 13.9	436.5 ± 132.2	427.0 ± 112.0
C_{max} (μg/ml)	10.2 ± 2.4	11.5 ± 2.7	2.0 ± 0.6	2.2 ± 0.7	5.4 ± 4.5	5.54 ± 1.3	31.8 ± 4.2	31.0 ± 4.1
T_{max} (h)	2.1 ± 1.0	2.3 ± 0.7	5.6 ± 2.1	5.3 ± 1.1	1.3 ± 0.5	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.5
$T_{1/2}$ (h)	4.9 ± 1.7	4.3 ± 0.8	–[a]	–[a]	3.3 ± 1.5	3.3 ± 1.4	8.6 ± 2.5	8.6 ± 2.0

All the values are given as mean ± S.D. ($n = 22$).
[a] As des-acetyl rifampicin is the metabolite its elimination half-life was not calculated.

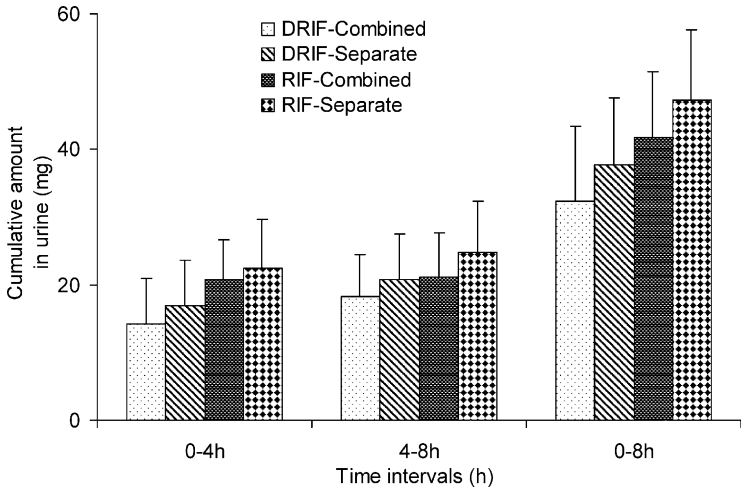

Fig. 2. Cumulative amounts of rifampicin and des-acetyl rifampicin excreted in urine at different time intervals. *Y*-error bars indicate S.D., $n = 22$. Abbreviations: RIF: rifampicin, DRIF: des-acetyl rifampicin.

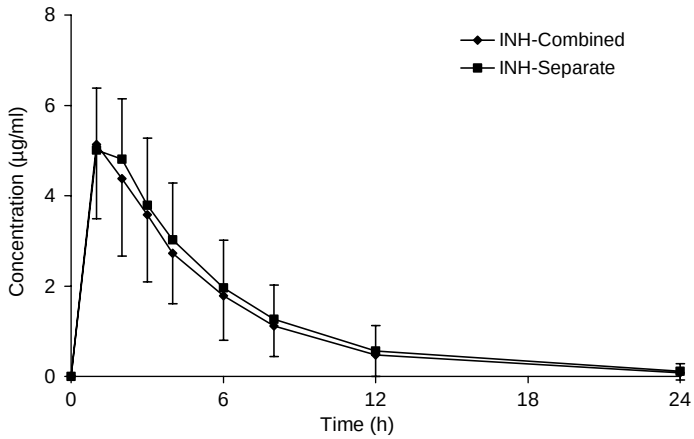

Fig. 3. Concentration time profiles of isoniazid from plasma when given as combined and separate formulations. *Y*-error bars indicate S.D., $n = 22$. Abbreviation: INH: isoniazid.

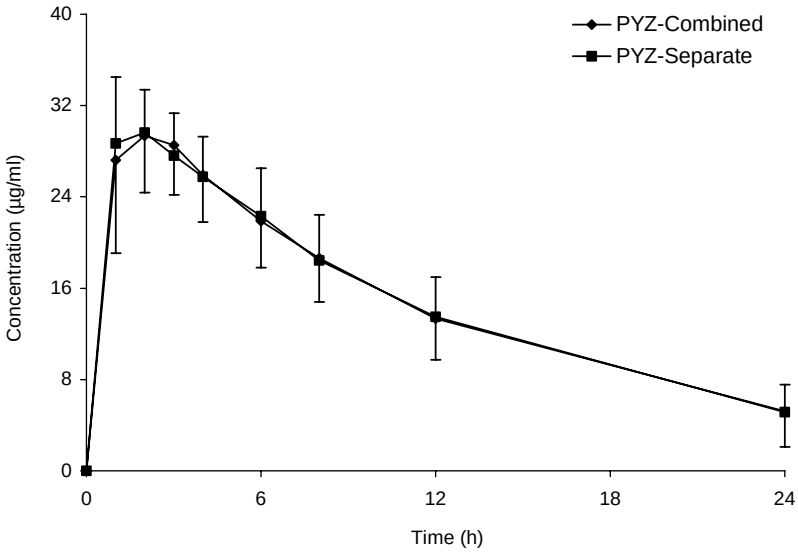

Fig. 4. Concentration time profiles of pyrazinamide from plasma when given as combined and separate formulations. *Y*-error bars indicate S.D., $n = 22$. Abbreviation: PYZ: pyrazinamide.

and mean $AUC_{0–8}$ for FDC formulations was 51.3 and 57.6 μg h/ml, respectively. Further, C_{max} of rifampicin obtained from these formulations was above 10 μg/ml, which is well above the therapeutic concentration and also is comparable to other studies conducted at NIPER at the dose of 600 mg of rifampicin (Panchagnula et al., 1999a, 2000). Lower T_{max} values of 2.05 and 2.32 h in combined and separate formulations indicate that rifampicin was rapidly absorbed from both the formulations. Rifampicin from FDC formulation was bioequivalent to separate formulations with power of the test above 95% (Table 2). Further, as evident from Table 2, there was no effect of reduced sampling time on the bioavailability of rifampicin. The active metabolite of rifampicin also exhibited similar pharmacokinetics from FDC and separate formulations (Fig. 1 and Table 1) and also passed the bioequivalence test. In addition, as can be seen from Fig. 2, urinary recovery of rifampicin as well as desacetyl rifampicin from both formulations is similar in time intervals of 0–4, 4–8 and 0–8 h. For urinary data, amount of rifampicin excreted in 0–4 h and 0–8 h are within the limits while 4–8 h is outside the limits. Rifampicin in urine does not appear until T_{max} is achieved, which is 2.05 and 2.32 h for combined and separate formulation, respectively. As T_{max} is differing, amount excreted after T_{max} is not uniform and hence limits of 4–8 h are outside the limits. Although, bioequivalence is successfully determined from urinary excretion data, in the present study, cumulative amount excreted versus time plot was not prepared because of the limited urinary sampling in the WHO recommended protocols (only two samples of 0–4 and 4–8 h) and hence urinary data was not included in the decision of bioequivalence (Ritschel and Kearns, 1999).

Concentration time profiles of isoniazid and pyrazinamide from FDC formulations were exactly superimposable to that of separate formulations (Figs. 3 and 4) due to comparable pharmacokinetic parameters and less variations in the absorption (Table 1). Both isoniazid and pyrazinamide were rapidly absorbed from GI tract as indicated by T_{max} at first sampling point in many volunteers and their plasma concentrations were within the therapeutic limits. Both these drugs were bioequivalent to separate counterparts. Bioequivalency of both rifampicin and isoniazid indicate that in vitro interaction is of clinical insignificance. As seen from Table 1, rifampicin and isoniazid are rapidly absorbed and hence in vivo interaction of these drugs is minimized because of their highly permeable nature.

Table 2
Pharmacokinetic parameters of rifampicin, des-acetyl rifampicin, isoniazid and pyrazinamide for the bioequivalence assessment from four drugs FDC formulation

Parameter	Lower limit	Upper limit
Pharmacokinetic parameters of RIF bioequivalence in plasma samples		
AUC_{0-8}	0.83	0.99
AUC_{0-24}	0.86	1.01
AUC_{0-inf}	0.86	1.01
C_{max}	0.81	0.96
Pharmacokinetic parameters of DRIF bioequivalence in plasma samples		
AUC_{0-8}	0.82	1.02
AUC_{0-24}	0.84	1.01
AUC_{0-inf}	0.87	1.05
C_{max}	0.82	1.01
Pharmacokinetic parameters of INH bioequivalence in plasma samples		
AUC_{0-8}	0.89	1.00
AUC_{0-24}	0.84	0.99
AUC_{0-inf}	0.83	0.99
C_{max}	0.91	1.03
Pharmacokinetic parameters of PYZ bioequivalence in plasma samples		
AUC_{0-8}	0.99	1.02
AUC_{0-24}	0.97	1.02
AUC_{0-inf}	0.97	1.05
C_{max}	1.00	1.06
Pharmacokinetic parameters of RIF bioequivalence in urine		
Amt. 0–4 h	0.85	1.04
Amt. 4–8 h	0.74	1.00
Amt. 0–8 h	0.81	1.00
Pharmacokinetic parameters of DRIF bioequivalence in urine		
Amt. 0–4 h	0.66	0.93
Amt. 4–8 h	0.77	1.01
Amt. 0–8 h	0.74	0.97

Bioequivalence criteria: 0.80–1.25.

4. Conclusions

The given four drug FDC formulation (Forecox Trac) was bioequivalent to rifampicin, isoniazid and pyrazinamide when compared to loose combination of separate formulations at the same dose levels. As this formulation was according to WHO recommended dose ratios, the treatment with FDC with the proven quality ensures therapeutic efficacy all the components thereby reducing the risk of monotherapy and emergence of drug resistance.

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