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Assessment of bioequivalence of rifampicin, isoniazid and pyrazinamide in a four drug fixed dose combination with separate formulations at the same dose levels

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Abstract

Tuberculosis (TB) needs treatment with three to five different drugs simultaneously, depending on the patient category. These drugs can be given as single drug preparations or fixed dose combinations (FDCs) of two more drugs in a single formulation. World Health Organization and International Union against Tuberculosis and Lung Disease (IUATLD) recommend FDCs only of proven bioavailability. The relative bioavailability of rifampicin (RIF), isoniazid (INH) and pyrazinamide (PYZ) was assessed on a group of 13 healthy male subjects from a four drug FDC versus separate formulations at the same dose levels. The study was designed to be an open, crossover experiment. A total of nine blood samples each of 3 ml volume were collected over a period of 24-h. The concentrations of RIF, its main metabolite desacetyl RIF (DRIF), INH and PYZ in plasma were assessed by HPLC analysis. Pharmacokinetic parameters namely AUC_{0-24}, $AUC_{0\text{-inf}}$, C_{max}, T_{max}, were calculated and subjected to different statistical tests (*Hauschke* analysis, two way ANOVA, normal and log transformed confidence interval) at 90% confidence interval. In addition, elimination rate constant (K_{el}) and absorption efficiencies for each drug were also calculated. It was concluded that four drugs FDC tablet is bioequivalent for RIF, INH and PYZ to separate formulation at the same dose levels. © 2002 Published by Elsevier Science B.V.

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

1. Introduction

All over the world, tuberculosis (TB) remains a major public health problem particularly in the developing countries. It is estimated that presently, there are more than 100 million cases of TB around the globe with 9–10 million cases being added each year (Ahlburg, 2000). Since the

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control measures for TB such as BCG vaccination and chemoprophylaxis seem to be unsatisfactory, treatment with anti-TB drugs becomes the only available option. The key to controlling TB with present tools is rapid detection and cure of infectious cases. But, in recent years treatment of TB has been threatened by increasing number of patients with drug resistant TB. As suggested by World Health Organization (WHO), treatment of TB and drug resistant cases requires multi-drug therapy, comprising of initial intensive phase with three to four first line anti-TB drugs for 2 months and continuation phase with two drugs for next 4 months (Maher et al., 1997). Though named Short Course Chemotherapy, for the patients, this treatment regimen is difficult to follow, as patients have to consume a large number of tablets, which is a common cause for non-compliance. It can be anticipated that non-optimal application of these short course regimens will result in the deterioration of their therapeutic potential.

The concept of fixed dose combination (FDC) aroused from the fact that TB always requires multi-drug therapy. FDC is a combination of two or more first-line anti-TB drugs in a single formulation at a fixed proportion. Thus, FDC is a simple approach to deliver the correct number of drugs at the right dosage as all the necessary drugs are combined in a single tablet. The other inherent advantages associated with FDCs are: reduced risk of emergence of drug resistant strains, lower cost of treatment, less risk of medication errors, simplified drug supply management, shipping and distribution, simplification and effective implementation of DOTS (Laing et al., 1999; Blomberg et al., 2001). Furthermore, many low-income countries have shown that, by using available tools both widely and wisely, TB deaths can be reduced five-fold. In this backdrop, FDCs assume importance as a potential strategy for TB treatment.

However, a major concern with widespread use of FDCs is quality of these dosage forms. Inadequate rifampicin (RIF) bioavailability has been reported from some FDCs, the use of such substandard FDCs will result in drug resistant TB and treatment failure (Ellard and Fourie, 1999; Pillai et al., 1999). For this reason, International Union Against Tuberculosis and Lung Disease (IUATLD) and WHO recommend the use of FDCs but only those of proven bioavailability (IUATLD/WHO, 1994) while they also have developed a simplified screening protocol for testing of RIF bioavailability (Fourie et al., 1999).

In this regard, our laboratory is one of the two reference laboratories of the world accredited by WHO for evaluation of FDCs of anti-tubercular drugs. Hence, present study was conducted to investigate the bioequivalence of four drugs FDC formulation of RIF, isonaizid (INH) and pyraznamide (PYZ) and ethambutol (ETB) against separate formulations at the same dose levels.

2. Materials and methods

2.1. Materials

RIF, INH, PYZ, ETB hydrochloride and rifapentine were kindly supplied by Lupin Laboratories Ltd. Desacetyl RIF (DRIF) was a gift sample from Dr Gordon Ellard, UK. All other reagents were either of HPLC or AR grade procured from Loba Chemie, Mumbai, India and Mallinckrodt, France. Ultra pure water prepared by reverse osmosis was filtered through 0.45 μm membrane filter and used in all the experiments.

2.2. Instruments

For analysis, Waters HPLC system (Milford, MA, USA) consisting of two 515 pumps, 717 plus autosampler and 2487 dual λ, absorbance detector was used. MILLENNIUM^{32} software (version 3.05.01) was used for data acquisition and processing. Other instruments used include Beckman DU® 640i spectrophotometer (Fullerton, CA, USA), Electrolab tablet dissolution tester (USP XXIII; Mumbai, India), 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), Brand autopipettes from E. Merck (Mumbai) and mi-

crolitre syringes from Hamilton (Bonaduz, Switzerland).

2.3. In vitro dissolution studies

In order to judge the quality of formulations and for better prediction of in vivo performance of test formulations dissolution studies were conducted for both the combined and separate formulations (USP 24-NF19, 2000).

2.3.1. Combined formulation

Dissolution study of FDC tablets was performed with USP apparatus 2 at 75 rpm ($n = 5$). The dissolution medium was simulated gastric fluid without pepsin maintained at 37 °C. At 0, 10, 20, 30 and 45 min, 5 ml of dissolution sample was withdrawn and replaced with an equal volume of fresh medium. Samples were analyzed by UV spectrophotometer at 475 nm for RIF and by HPLC at 267 nm for INH and PYZ.

2.3.2. Separate formulations

Dissolution studies of the separate formulations were performed with 900 ml of dissolution medium (37 °C, 0.1 N HCl for RIF and 900 ml of distilled water for INH, PYZ and ETB; $n = 5$) using USP apparatus 1 at 100 rpm. Sampling points were at 0, 10, 20, 30 and 45 min with replacement of fresh medium. The samples were analyzed at 475, 262 and 269 nm for RIF, INH and PYZ, respectively.

2.4. Bioequivalence trial

2.4.1. Experimental design

The study was designed as an open, crossover experiment on a group of 14 (considering two dropouts) healthy volunteers after getting the routine approval from the NIPER Ethical Committee (Schedule, 1999). The ethical committee is equivalent to the 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 and routine urine analysis. After screening, fourteen healthy subjects were selected. The scope of the study was explained to all the subjects and each one signed an informed consent form before onset of the study.

2.4.3. Dosing schedule

On each experimental session, formulations were swallowed on an empty stomach after overnight fast with a glass of water (approximately 200 ml). A light breakfast and lunch was provided after 2 and 6 h, respectively. A group of fourteen healthy subjects received either two FDC tablets (the test formulation containing 225 mg of RIF, 150 mg of INH, 750 mg of PYZ and 400 mg of ETB) or standard separate daily drug formulations (RIF tablet containing 450 mg RIF, INH tablet having 300 mg of INH, PYZ tablet having 750 mg of PYZ and ETB tablet having 800 mg of ETB). The administration sequence was randomized in order to reduce the sequence and period effect.

2.4.4. Collection of blood samples

Venous blood samples (3 ml) were collected in heparinized tubes using indwelling catheter at 0 h (shortly before the ingestion of drugs), 1, 2, 3, 4, 6, 8, 12 and 24 h after the ingestion of drug doses. After collection, blood samples were immediately centrifuged at 8000 rpm for a period of 20 min. Plasma was separated into tubes containing ascorbic acid (0.5 mg/ml of plasma) to prevent oxidative degradation of RIF and stored at -20 °C till analysis.

2.5. Bioanalytical work

2.5.1. Analysis of RIF and DRIF

RIF and DRIF were analyzed by high performance liquid chromatography (HPLC) method developed in this laboratory (Panchagnula et al., 1999a). Rifapentine was used as an internal standard and separation was achieved on Nova Pak C_{18} (250×4.6 mm i.d., 4 μm) column. Mobile phase composition 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 INK, PYZ, ETB and their metabolites within a maximum run time of 20 min.

2.5.2. Analysis of INH and PYZ

Analysis of INH and PYZ from plasma samples was done by a method developed in this laboratory (Agrawal, 1999). Analytical column used was reversed phase Spherisorb C_8 (250 × 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, the peaks of INH hydrazone and PYZ were well resolved without the interference of any other drug or its metabolites within a run time of 30 min. Analysis of INH 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.

2.6. Calculation of pharmacokinetic parameters

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. k_{ef} (elimination rate constant), $t_{1/2}$ (half life of the drug) and absorption efficiencies were also calculated.

2.7. Statistical analysis

To assess the bioequivalence, various pharmacokinetic parameters were evaluated by non-parametric *Hauschke* analysis (Hauschke et al., 1990), parametric two way ANOVA (Fourie et al., 1999; Panchagnula et al., 1999b), normal and log transformed confidence intervals at 90% confidence interval.

3. Results

3.1. In vitro dissolution studies

Results of dissolution studies have shown that both, combined and separate formulations have desired dissolution profiles for all the four drugs i.e. the drug release was not less than 75% in 45 min. There was no difference between the dissolution of individual components of FDC compared with separate formulations that can affect drugs behavior in vivo.

3.2. Clinical study

From the symptom checklist of both the periods, no serious or unexpected side effects were observed except a few cases of mild headache and heavy head that was common with both combined and separate formulations. Hence, there were no untoward effects of FDCs over separate formulations.

Due to some reasons one of the volunteer (vol. code V-7) dropped out in the second period therefore, the data of 13 volunteers has been considered for the statistical evaluation.

3.3. Pharmacokinetic and statistical results

The mean concentrations of RIF and DRIF are plotted as a function of time and shown in Fig. 1. Also, Figs. 2 and 3 show concentration-time profiles of INH and PYZ, respectively. The various pharmacokinetic parameters for RIF and DRIF such as AUC_{0-24}, AUC_{0-inf}, C_{max} and T_{max} for each volunteer are listed in Tables 1 and 2, respectively. The K_{el} and $t_{1/2}$ were also calculated and listed in the respective tables. The absorption efficiencies for RIF were calculated for individual subjects according to the formula given elsewhere and listed in Table 1, which is an indication of any absorption, problems encountered and thereby leading to decreased bioavailability of drugs (Panchagnula et al., 1999b). Similarly, Tables 3 and 4 enlist pharmacokinetic parameters and absorption efficiencies of INH and PYZ, respectively. Upper and lower limits of bioequivalence obtained after statistical analysis of three drugs are given in Table 5. Bioequivalence limits for T_{max} are not given in Table 5 as T_{max} is a secondary parameter for assessment of bioequivalence and is mostly affected by the truncated sampling procedure adopted.

The mean C_{max} values for RIF were 7.09 and 6.61 μg/ml for combined and separate formula-

Table 1
Pharmacokinetic parameters for comparison of combined and separate formulations for RIF bioequivalence

Pharmacokinetic parameters of RIF														
Volunteer code	Sequence	Combined							Separate					
		AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg h/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)	Absorption efficiencies	AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg h/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)	Absorption efficiencies
V-1	CS	27.20	29.59	3.08	4	0.1168	5.93	0.61	33.26	37.53	4.05	3	0.1024	6.77	0.67
V-2	CS	49.97	49.97	7.49	2	0.1494	4.64	1.06	29.82	29.82	4.34	2	0.1918	3.61	0.81
V-3	SC	51.08	52.75	5.09	3	0.1496	4.63	0.96	63.18	65.18	5.95	1	0.1438	4.82	1.15
V-4	SC	31.96	31.96	4.12	2	0.1672	4.15	0.74	29.55	29.55	4.08	2	0.1933	3.59	0.79
V-5	SC	42.27	44.02	5.00	4	0.1494	4.64	0.88	47.25	47.96	7.68	2	0.1805	3.84	1.15
V-6	CS	32.36	32.36	5.61	2	0.2661	2.60	1.15	27.06	27.06	4.93	3	0.3105	2.23	1.12
V-8	CS	46.88	46.88	5.99	1	0.1673	4.14	1.01	37.93	39.46	4.60	4	0.1835	3.78	0.93
V-9	CS	36.89	40.14	3.83	2	0.1072	6.46	0.61	36.08	38.78	4.32	3	0.1229	5.64	0.68
V-10	SC	43.06	43.06	6.41	3	0.1494	4.64	0.99	31.29	31.29	5.56	3	0.1936	3.58	0.93
V-11	CS	41.50	43.16	4.49	3	0.1319	5.25	0.94	37.87	39.34	4.13	3	0.1380	5.02	0.89
V-12	SC	40.55	41.80	5.56	2	0.1506	4.60	0.92	51.50	52.88	6.38	3	0.1603	4.32	1.24
V-13	SC	44.99	44.99	8.77	2	0.1950	3.55	1.23	43.46	43.46	6.58	2	0.1748	3.96	1.06
V-14	CS	45.85	45.85	6.61	2	0.1913	3.62	1.23	49.11	50.64	7.67	2	0.1705	4.07	1.21
	Mean	41.12	42.04	5.54	2.46	0.16	4.53	0.95	39.80	41.00	5.41	2.54	0.17	4.25	0.97
	S.D.	7.22	6.98	1.55	0.88	0.04	1.00	0.20	10.58	10.95	1.33	0.78	0.05	1.12	0.20

Table 2
Pharmacokinetic parameters for comparison of combined and separate formulations for DRIF bioequivalence

Pharmacokinetic parameters of DRIF													
Volunteer code	Sequence	Combined						Separate					
		AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg h/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)	AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)
V-1	CS	9.66	11.06	0.78	6	0.0785	8.83	10.51	11.05	1.05	4	0.1912	3.62
V-2	CS	20.15	20.15	1.69	4	0.0853	8.13	9.85	9.85	0.96	6	0.1501	4.62
V-3	SC	17.15	18.04	1.52	8	0.1414	4.90	23.66	24.90	1.72	4	0.1527	4.54
V-4	SC	9.13	9.13	1.04	4	0.1615	4.29	9.91	9.91	1.21	4	0.1909	3.63
V-5	SC	13.35	14.33	1.56	4	0.1298	5.34	15.25	16.15	1.47	6	0.1412	4.91
V-6	CS	12.95	12.95	1.45	4	0.2248	3.08	8.63	8.63	1.18	4	0.2310	3.00
V-8	CS	27.23	28.17	2.65	4	0.1758	3.94	16.12	16.83	1.53	4	0.1424	4.86
V-9	CS	7.63	7.63	0.65	3	0.1094	6.33	7.18	7.18	0.81	6	0.1680	4.12
V-10	SC	16.64	16.64	1.68	3	0.1624	4.27	11.59	11.59	1.33	3	0.1160	5.97
V-11	CS	8.94	8.94	0.84	6	0.1162	5.96	7.96	7.96	0.71	6	0.1129	6.14
V-12	SC	14.52	14.52	1.43	3	0.1553	4.46	23.30	25.23	2.04	4	0.1269	5.46
V-13	SC	16.39	16.39	1.80	2	0.2029	3.42	18.06	18.06	1.58	3	0.1845	3.76
V-14	CS	9.66	9.66	1.50	4	0.0795	8.71	12.74	12.74	1.56	4	0.2122	3.27
	Mean	14.11	14.43	1.43	4.23	0.14	5.51	13.44	13.85	1.32	4.46	0.16	4.45
	S.D.	5.51	5.66	0.53	1.59	0.05	1.96	5.51	6.03	0.38	1.13	0.04	1.00

Table 3
Pharmacokinetic parameters for comparison of combined and separate formulations for INH bioequivalence

Pharmacokinetic parameters of INH															
Volunteer code	Sequence	Combined							Separate						
		AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg/ml)	T_{max} (h)	K_{el} (l/h)	$t_{1/2}$ (h)	Absorption efficiencies	AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg/ml)	T_{max} (h)	K_{el} (l/h)	$t_{1/2}$ (h)	Absorption efficiencies
V-1	CS	7.60	7.60	1.55	3	0.1359	5.10	0.27	10.74	10.74	1.70	2	0.2435	2.85	0.69
V-2	CS	24.39	24.39	5.25	1	0.2758	2.51	1.44	25.46	25.46	5.60	1	0.2486	2.79	1.35
V-3	SC	34.84	37.21	3.94	2	0.1218	5.69	0.83	36.49	38.77	7.32	1	0.1268	5.46	0.90
V-4	SC	18.61	18.61	4.49	1	0.2232	3.10	0.86	15.90	15.90	4.16	1	0.2900	2.39	0.95
V-5	SC	31.87	31.87	4.75	2	0.2121	3.27	1.35	37.14	38.35	5.36	1	0.1527	4.54	1.17
V-6	CS	9.04	9.04	2.85	2	0.3257	2.13	0.59	9.27	9.27	2.09	1	0.3731	1.86	0.69
V-8	CS	15.60	15.60	4.26	1	0.2487	2.79	0.75	15.54	16.37	3.82	2	0.1484	4.67	0.47
V-9	CS	29.21	29.21	5.02	1	0.1974	3.51	1.23	34.71	36.47	4.78	2	0.1356	5.11	1.06
V-10	SC	17.72	17.72	4.80	1	0.3487	1.99	1.42	19.19	19.19	5.24	1	0.3492	1.98	1.54
V-11	CS	23.51	24.89	3.58	2	0.1306	5.31	0.80	23.29	24.40	3.28	3	0.1323	5.24	0.80
V-12	SC	13.31	15.03	2.69	1	0.1081	6.41	0.36	15.43	16.00	3.65	1	0.1566	4.43	0.55
V-13	SC	24.97	24.97	5.16	1	0.1582	4.38	0.83	24.80	24.80	3.49	2	0.2500	2.77	1.30
V-14	CS	31.04	31.04	4.45	1	0.1570	4.41	1.02	32.32	32.32	5.34	2	0.1966	3.52	1.33
	Mean	21.67	22.09	4.06	1.46	0.20	3.89	0.90	23.10	23.70	4.29	1.54	0.22	3.66	0.99
	S.D.	8.82	9.04	1.11	0.66	0.08	1.43	0.38	9.71	10.22	1.53	0.66	0.08	1.30	0.34

Table 4
Pharmacokinetic parameters for comparison of combined and separate formulations for PYZ bioequivalence

Volunteer code	Sequence	Pharmacokinetic parameters of PYZ													
		Combined							Separate						
		AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg h/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)	Absorption efficiencies	AUC_{0-24} (μg h/ml)	$AUC_{0\text{-inf}}$ (μg h/ml)	C_{max} (μg/ml)	T_{max} (h)	K_{el} (1/h)	$t_{1/2}$ (h)	Absorption efficiencies
V-1	CS	276.18	355.28	20.85	4	0.0669	10.36	1.25	282.58	346.49	21.10	3	0.0729	9.51	1.33
V-2	CS	313.30	360.33	27.48	1	0.0862	8.04	1.33	312.64	355.12	28.50	1	0.0879	7.88	1.33
V-3	SC	330.36	453.80	25.42	2	0.0572	12.11	0.95	324.51	395.57	28.83	1	0.0719	9.63	1.04
V-4	SC	312.04	365.73	29.12	1	0.0827	8.38	1.25	318.16	384.50	27.09	1	0.0758	9.14	1.20
V-5	SC	283.00	305.45	26.34	4	0.1111	6.24	1.36	319.11	355.87	26.32	2	0.0932	7.44	1.33
V-6	CS	298.53	324.80	29.76	2	0.1043	6.64	1.36	265.36	293.46	27.05	3	0.1004	6.90	1.18
V-8	CS	319.41	359.89	26.84	1	0.0890	7.78	1.24	319.31	372.46	28.26	2	0.0821	8.44	1.18
V-9	CS	299.30	334.36	24.41	2	0.0920	7.53	1.31	286.96	315.93	26.07	2	0.1003	6.91	1.35
V-10	SC	221.38	227.75	25.00	1	0.1418	4.89	1.49	236.97	259.29	24.84	1	0.1031	6.72	1.23
V-11	CS	217.41	243.00	20.88	2	0.0947	7.32	1.14	238.76	262.51	19.90	2	0.1002	6.92	1.30
V-12	SC	268.03	291.64	23.05	2	0.1030	6.73	1.32	276.61	313.54	25.73	1	0.0897	7.73	1.24
V-13	SC	339.07	416.20	30.59	1	0.0734	9.44	1.28	336.74	427.76	27.16	2	0.0669	10.35	1.20
V-14	CS	317.97	385.83	27.33	1	0.0745	9.30	1.21	320.93	396.25	27.69	2	0.0703	9.85	1.17
	Mean	292.00	340.31	25.93	1.85	0.09	8.06	1.27	295.28	344.5	26.04	1.77	0.09	8.26	1.24
	S.D.	38.21	63.49	3.10	1.07	0.02	1.90	0.13	33.25	52.61	2.72	0.73	0.01	1.29	0.09

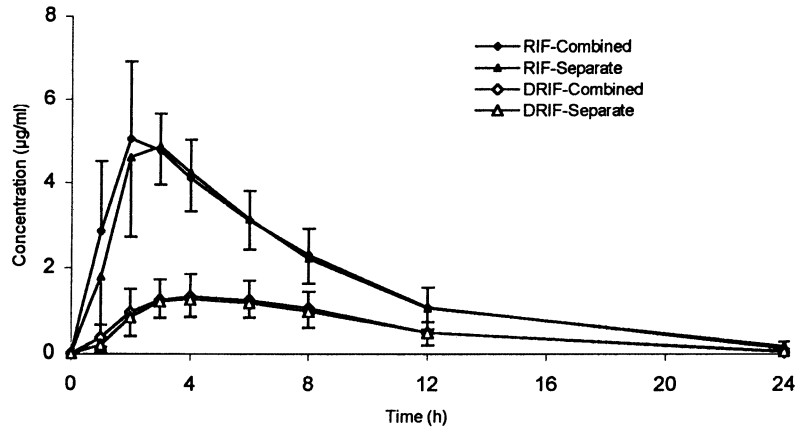

Fig. 1. Concentration time profiles of RIF and DRIF from plasma when given as combined and separate formulations.

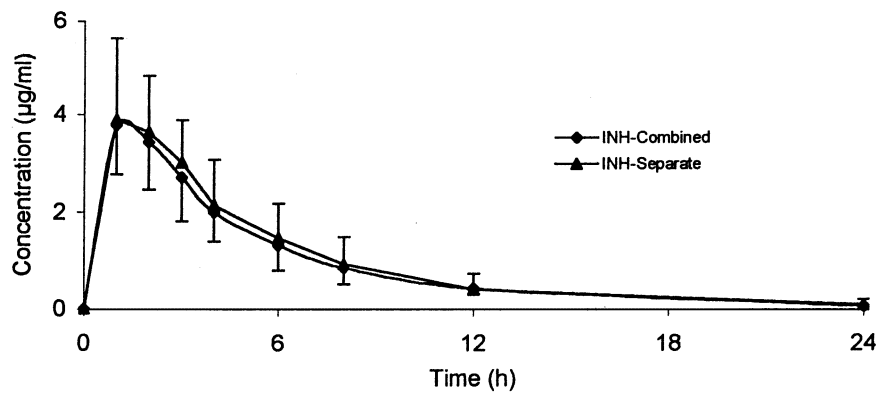

Fig. 2. Concentration time profiles of INH from plasma when given as combined and separate formulations.

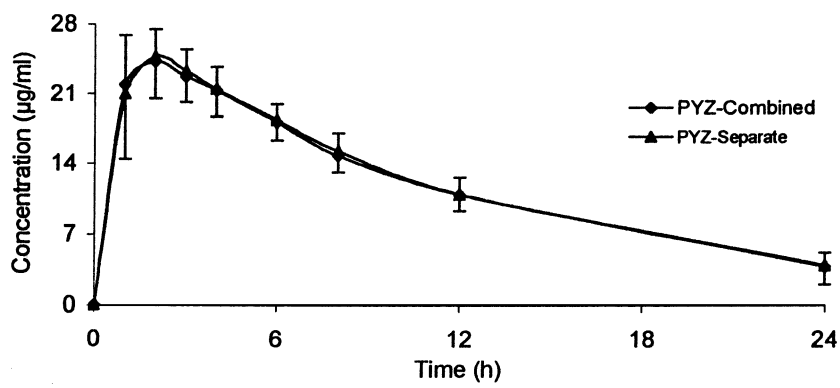

Fig. 3. Concentration time profiles of PYZ from plasma when given as combined and separate formulations.

Table 5
Pharmacokinetic parameters of RIF, DRIF, INH and PYZ for the bioequivalence assessment from four drugs FDC

Pck parameter	Non-parametric		Parametric		Normal CI		Log transformed CI	
	LL	UL	LL	UL	LL	UL	LL	UL
Pharmacokinetic parameters of RIF bioequivalence								
AUC_{0-24}	0.92	1.16	0.93	1.14	0.95	1.20	0.94	1.17
$AUC_{0\text{-inf}}$	0.92	1.16	0.92	1.13	0.94	1.19	0.93	1.17
C_{max}	0.87	1.20	0.88	1.17	0.91	1.19	0.89	1.16
Pharmacokinetic parameters of DRIF bioequivalence								
AUC_{0-24}	0.84	1.24	0.86	1.23	0.91	1.33	0.89	1.26
$AUC_{0\text{-inf}}$	0.85	1.24	0.85	1.23	0.92	1.33	0.88	1.26
C_{max}	0.91	1.24	0.92	1.23	0.93	1.27	0.92	1.22
Pharmacokinetic parameters of INH bioequivalence								
AUC_{0-24}	0.90	0.99	0.88	0.99	0.89	1.00	0.88	0.99
$AUC_{0\text{-inf}}$	0.88	0.98	0.86	0.99	0.88	0.99	0.88	0.99
C_{max}	0.83	1.11	0.79	1.09	0.87	1.12	0.85	1.10
Pharmacokinetic parameters of PYZ bioequivalence								
AUC_{0-24}	0.96	1.01	0.96	1.02	0.96	1.02	0.96	1.02
$AUC_{0\text{-inf}}$	0.94	1.03	0.95	1.03	0.94	1.03	0.94	1.02
C_{max}	0.94	1.04	0.96	1.04	0.96	1.03	0.96	1.03

LL, lower limit; UL, upper limit for bioequivalence. Bioequivalence criteria, LL, 0.8; UL, 1.25.

tions, respectively. All the volunteers showed T_{max} values of 1–3 h for both test and standard formulations (Table 1). It is evident from the Table 5 that in case of RIF and DRIF, all the three primary pharmacokinetic parameters were found to be within the limits of bioequivalence when compared by non-parametric *Hauschke* analysis or parametric two way ANOVA. In addition, the mean absorption efficiencies of both combined and separate formulations were 0.95 and 0.97, respectively, suggesting no problem in the absorption from any of the formulations.

In case of INH and PYZ, all the three primary pharmacokinetic parameters were found to be within the limits of bioequivalence when compared by any of the statistical tests (Table 5).

4. Discussion

Tuberculosis is a major health problem in the developing countries like India, which has the maximum pool of TB patients. As of now the only available treatment lies in effective utilization of the available anti-TB drugs. However, the emergence of resistant strains has come as a major 'bottleneck' in the treatment of TB. Combination of drugs can effectively counter this problem that led to the concept of FDCs. At the same time, it is very important to ensure that the bioavailability of the drugs combined in the FDCs is not compromised. This is particularly true for RIF where there are conflicting reports on the relative bioavailability from FDCs compared with separate formulations. The exact cause of the compromised RIF bioavailability from some of the formulations is yet not clear and speculative. Therefore, the WHO and IUTALD recommend only those FDCs of proven bioavailability. NIPER, which is one of the two centers in the world, accredited by WHO for assessing the bioavailability of anti-TB drugs, found that the four drug FDC bioequivalent to separate formulations in terms of all pharmacokinetic parameters. The absence of any negative interaction between the components of this four drugs FDC indicates that such formulation can be used in TB control programs without compromising the therapeutic potential of these drugs.

5. Conclusion

All the primary parameters of RIF, DRIF, INH and PYZ for the bioequivalence assessment are within the acceptable limits of 0.80–1.25. Therefore, it is concluded that combined formulation is bioequivalent to separate formulations of RIF, INH and PYZ at the same dose levels. The use of such formulations with proved bioavailability will help in effective control and management of TB.

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