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Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation

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Abstract

The present study describes comparative bioavailability of rifampicin (RIF) after administration of a single component RIF (450 mg) capsule and rifampicin-isoniazid (RIF-INH) (450 + 300 mg) fixed dose combination (FDC) capsule formulations. Six healthy male volunteers participated in a single dose, two treatment, two period, cross-over study. A sensitive, specific and accurate HPTLC method was developed, validated and employed for estimation of RIF and its major active metabolite, 25-Desacetylrifampicin (25-DAR) levels, in urine. Using the urinary excretion data various pharmacokinetic parameters: AUC_{0-24}, $AUC_{0-\infty}$, cumulative amount excreted in 24 h, peak excretion rate, etc. for both RIF and 25-DAR were calculated and compared statistically (ANOVA, 90% confidence interval for ratio). Significant decrease in the bioavailability (~ 32% as RIF and ~ 28% as 25-DAR) of RIF from FDC capsules was observed. The present bioavailability study confirms our serious doubts about the stability of RIF in presence of INH in acidic environment of stomach, which probably is the main factor responsible for the reduced bioavailability of RIF from RIF-INH combination formulations. This study underlines the fact that there is an urgent need to reconsider the formulation of the FDC product in order to minimize or avoid the decomposition of RIF in gastrointestinal tract.

Keywords: Rifampicin; Isoniazid; 25-Desacetylrifampicin; HPTLC; Fixed dose combination (FDC); Bioavailability; Urinary excretion

1. Introduction

Tuberculosis is believed to claim 2 million lives a year all over the world. Countries with poor healthcare systems suffer the most. Emergence of multi-drug resistant (MDR) strains of *M. tuberculosis* and a co-infection with AIDS prompted WHO in March 1993 to declare tuberculosis as 'Global health emergency' (Annon, 1997). Some of the strains have developed resistance to drugs like rifampicin, isoniazid and streptomycin. The death rate for MDR-TB in immunity suppressed patients can be as high as 80%.

Rifampicin (RIF), isoniazid (INH), pyrazinamide (PZ) and ethambutol (ETB) are the drugs of choice for treating tuberculosis. Fixed Dose Combination (FDC) of two, three or four drugs is a preferred dosage form for better patient compli-

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ance, efficient reduction in viable bacterial population and minimizing development of resistance to antitubercular drugs.

However, poor bioavailability of RIF from a number of dosage forms of RIF and its combination with INH continues to be a subject of much concern (Mouton et al., 1979; Doshi et al., 1986; Ellard et al., 1986; Acocella, 1989; Fox, 1990; Anonn, 1991). In 1994, World Health Organization (WHO) and International Union against Tuberculosis and Lung Disease (IUATLD) cautioned that antitubercular FDC formulations should be used only if the bioavailability of RIF has been demonstrated convincingly (IUATLD/WHO, 1994). A protocol has been published for testing of bioequivalence of RIF from FDC products (Fourie et al., 1999).

It has been suggested that probable factors responsible for the variation in bioavailability of RIF from different FDC formulations include particle size and crystalline form of the drug, manufacturing process and the excipients used (Boman et al., 1975; Pelizza et al., 1977; Khalil et al., 1984; Cavenaghi, 1989; Henwood et al., 2000). However, none of these studies explain, convincingly, the impairment of bioavailability of RIF from FDC formulations. RIF is known to hydrolyze to insoluble 3-Formyl rifamycin SV (3-FRSV) in acidic medium (Gallo and Radaelli, 1976; Prankerd et al., 1992) and the hydrolysis is accelerated significantly in presence of INH (Shishoo et al., 1999; Singh et al., 2000). The decomposition of RIF under these conditions varies from 8.5 to 50% in the time range corresponding to the gastric residence time in humans (15 min to 3 h) (Singh et al., 2000).

Our in vitro study has indicated that RIF, in presence of INH (as FDC), may undergo greater decomposition in acidic conditions of stomach, as compared to RIF alone when administered orally. Since, less amount of RIF will be available for absorption from FDCs as compared to RIF alone formulation, it should be reflected in the bioavailability of RIF from these two formulations.

It has been established that renal elimination of RIF is a function of its dose (Brechbuhler et al., 1978) and considerable amount of RIF (6–15% of the dose) and its major active metabolite-25-Desacetylrifampicin (25-DAR) (15% of the dose) are excreted in urine (USP DI, 1996). Renal elimination of RIF plus 25-DAR provides a reliable and much more convenient method of assessing RIF bioavailability than estimating area under the plasma concentration-time curve (Brechbuhler et al., 1978; Ellard et al., 1986). Besides, urine provides a non-invasive sample collection method and urinary excretion data has been successfully used to determine comparative bioavailability or bioequivalence of the formulations in several studies (Adams et al., 1979; Straughn et al., 1979; Adriana et al., 2000).

The present study assesses comparative bioavailability of RIF in terms of urinary excretion of RIF and 25-DAR, after administration of market samples of 'RIF-INH FDC capsules' and 'capsules containing only RIF'. A highly sensitive and specific HPTLC method was developed, validated and employed to determine urinary excretion of RIF and 25-DAR.

2. Experimental

2.1. HPTLC method for estimation of RIF and 25-DAR in urine

2.1.1. Instruments

CAMAG-HPTLC system consisting of CAMAG Linomat IV semiautomatic spotter device, CAMAG twin-trough TLC chamber, CAMAG-TLC scanner 3 and CAMAG CATS 4 software and Hamilton syringe (100 μl) was used.

2.1.2. Materials

Analytically pure RIF powder was gifted by Themis Laboratories, Mumbai. 25-DAR was synthesized in laboratory and characterized. All other chemicals, chloroform (JC's Chemicals, Vadodara), methanol (Ranbaxy, Delhi), anhydrous sodium sulphate, ascorbic acid (Samir Tech-chem, Ahmedabad), isopropyl alcohol (S.D. Fine Chemicals, Boisar) were of analytical reagent grade. TLC aluminum sheets pre-coated with silica gel 60 F_{254} (layer thickness 0.2 mm, 10×10 cm) (E. Merck, Germany) were used.

2.1.3. Preparation of stock solutions

2.1.3.1. Preparation of stock solutions of RIF and 25-DAR. The stock solutions of RIF and 25-DAR in methanol (1 mg/ml) were prepared by dissolving 10 mg of each drug separately. Ascorbic acid (5 mg) was added as an antioxidant.

2.1.3.2. Preparation of combined standard solution of RIF and 25-DAR. Aliquots of 0.9 ml of RIF stock solution and 0.9 ml of 25-DAR stock solution were mixed in a 10 ml volumetric flask and diluted up to the volume with methanol to obtain final concentration of 90 μg/ml RIF and 90 μg/ml 25-DAR.

2.1.4. Extraction of RIF and 25-DAR from urine

Drug-free urine or urine spiked with fixed aliquots of RIF or 25-DAR stock solutions or volunteer urine sample (5 ml) was taken in a stoppered glass tube (15 ml capacity) and sodium chloride (1 g) was added to it. It was mixed and the mixture was extracted with two portions of chloroform, 4 and 1 ml, by shaking the tube manually for 5 min, every time. Chloroform layer was separated and combined chloroform layer (4 ml) was dried over anhydrous sodium sulphate. Ascorbic acid (5 mg) was added to chloroform layer and it was stored at -20 °C until analyzed.

2.1.5. Chromatographic conditions

Chromatographic estimations were performed using pre-coated TLC plates under following conditions: Mobile phase, chloroform:methanol: isopropyl alcohol (85:10:10 v/v); chamber saturation time, 30 min; temperature, 25 ± 1 °C; migration distance, 35 mm; slit dimension, 3×0.3 mm; wavelength of detection, 475 nm; space between two bands, 4 mm; spraying rate, 10 s/μl.

2.1.6. Chromatographic analysis

Ten μl of combined standard solution of RIF and 25-DAR in methanol (90 μg/ml each) or fixed volumes of chloroform extracts, of drug-free urine/urine spiked with RIF and/or 25-DAR or volunteer urine samples, was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried and developed using mixture of chloroform:methanol:isopropyl alcohol (85:10:10 v/v) as the mobile phase at constant temperature (25 ± 1 °C). After development the plate was dried for 5 min. Photometric measurements were performed at 475 nm in the reflectance mode with CAMAG TLC scanner 3 connected to a computer running CATS 4 software incorporating the track optimization option. Quantitative determinations were carried out using the peak areas.

2.1.7. Calibration curve for standard RIF and 25-DAR

Aliquots of 0.4, 0.6, 0.8, 0.9 and 1.1 ml of the stock solution of RIF were mixed with equal volumes of stock solution of 25-DAR in different 10 ml volumetric flasks. Ascorbic acid (5 mg) was added into each flask and the mixtures were diluted with chloroform to get final concentration of 40, 60, 80, 90 and 110 μg/ml of RIF and 25-DAR.

Ten μl of each solution was spotted on TLC plate, the plate was developed, dried at room temperature and analyzed.

2.1.8. Calibration curve for RIF and 25-DAR spiked in urine

Equal volumes of 0.4, 0.6, 0.8, 0.9 and 1.1 ml of stock solutions of RIF and 25-DAR were mixed and diluted to 10 ml with drug/metabolite-free urine to get final concentrations of 40, 60, 80, 90 and 110 μg/ml of both RIF and 25-DAR. These solutions were extracted using chloroform as described earlier. Ten μl of each of the chloroform extracts was spotted on TLC plate and analyzed as above.

2.2. Validation of HPTLC method

2.2.1. Precision

The intra-day precision was determined by analyzing urine samples spiked with RIF and 25-DAR at 40, 80 and 110 μg/ml levels for three times on the same day. The inter-day precision was determined by analyzing urine samples spiked with RIF and 25-DAR at 40, 60, 80, 90 and 110 μg/ml levels daily for 5 days over a period of 1 week.

2.2.2. Repeatability of measurement of peak area

Ten μl of combined standard solution of RIF and 25-DAR (RIF 90 μg/ml + 25-DAR 90 μg/ml) was spotted on TLC plate and developed. The separated spots were scanned for seven times without changing position of plate and R.S.D. (% C.V.) for measurement of peak area was computed.

2.2.3. Repeatability of sample application

Ten μl of combined standard solution of RIF and 25-DAR (RIF 90 μg/ml + 25-DAR 90 μg/ml) was applied seven times on TLC plate by semiautomatic spotter, developed and analyzed as described above.

2.2.4. Accuracy

Accuracy of an analysis is determined by calculating systemic error involved. It was determined by calculating recovery of RIF and 25-DAR by standard addition method at different concentration levels.

2.2.5. Linearity

The linear response for RIF and 25-DAR was determined by analyzing corresponding standards five times for each concentration in the range of 40–110 μg/ml (400–1100 ng/spot).

2.2.6. Specificity

To confirm specificity of the proposed method; RIF, 25-DAR, 3-FRSV and rifampicin quinone were spiked in urine, extracted and analyzed. In addition, purity of chromatographic peaks corresponding to RIF and 25-DAR was confirmed by recording the spectra at peak start, peak apex and peak end positions of both the spots.

2.2.7. Stability of RIF and 25-DAR in urine

Stability of RIF and 25-DAR in urine under storage conditions (−20 °C, 7 days), was studied by analyzing spiked urine samples containing 40 and 110 μg/ml concentrations of both RIF and 25-DAR. The samples were analyzed on the 8th day for the amount of RIF and 25-DAR.

2.3. Comparative bioavailability study

2.3.1. Study protocol

Six healthy male volunteers, aged 22–25 years, weighing 50–70 kg, participated in the study. The written informed consent was obtained from all volunteers and protocol of the study was approved by the Local Ethical Committee. Bioavailability of RIF from RIF-INH FDC capsule (RIF 450 mg + INH 300 mg) was compared with that from RIF capsule (RIF 450 mg). The volunteers were fasted overnight and fasting was continued until 4 h post dose, however, free access to water was allowed. Each volunteer received single oral dose of capsule with 200 ml potable water, in an open randomized two treatment, two period, cross-over fashion with 10 days washout period between two treatments. No other drugs were taken by the volunteers 2 weeks before and until the end of the study.

Urine samples were collected before administration and 1, 2, 3, 4, 6, 8, 10, 12, 24 h after administration of the formulation. The urine output was measured, a representative sample ($\sim$10 ml) was transferred in test tube containing 5 mg of ascorbic acid, capped with aluminum foil and stored at −20 °C until analyzed. The samples were extracted and analyzed for amount of RIF and/or 25-DAR, after bringing them to room temperature, by the proposed HPTLC method. The respective amounts were calculated by fitting corresponding peak area in calibration curve equations for spiked RIF and 25-DAR.

2.3.2. Pharmacokinetic analysis

Various pharmacokinetic parameters viz. cumulative amount excreted over a period of 24 h, cumulative % drug/metabolite excreted (with respect to RIF dose), peak excretion rate $(dAU/dt)_{max}$, peak excretion time (t_{max}), were determined using urinary excretion data for both RIF and 25-DAR. Other parameters like area under the urinary excretion rate (untransformed and log transformed) versus mid-point time up to 24 h (AUC_{0-24}) and extrapolated up to infinite time ($AUC_{0-\infty}$), elimination rate constant (k_{el}) and elimination half life ($t_{1/2}$) were also calculated.

2.3.3. Statistical analysis

Various pharmacokinetic parameters, mainly AUC_{0-24}, $(dAU/dt)_{max}$ and cumulative drug/metabolite excreted in 24 h, were compared using different statistical tests. The parameters for RIF and 25-DAR obtained after administration of RIF-INH capsule were compared with that of RIF capsule by applying ANOVA and 90% confidence intervals for the ratio of means.

3. Results

3.1. Development of HPTLC method and validation

HPTLC method was selected owing to its simplicity, specificity and sensitivity. In the proposed study, because of high solubility of both the analytes, chloroform was selected as the solvent for extraction of RIF and 25-DAR from urine. Two times extraction and salting out of the analytes with sodium chloride further helped to improve the extraction efficiency. Overall average extraction efficiency for RIF and 25-DAR from urine was found to be 83 and 81%, respectively. Mobile phase consisting of chloroform:methanol: isopropyl alcohol (85:10:10 v/v) gave sharp, well resolved peaks of RIF and 25-DAR which were well separated (R_f of RIF $= 0.74 \pm 0.02$, R_f of 25-DAR $= 0.55 \pm 0.02$) (Fig. 1). The detection of RIF and 25-DAR was carried out at wavelength maxima, 475 nm.

The least square linear regression analysis of the peak area (y) versus concentration of RIF and 25-DAR in urine (x), obtained by assaying urine samples spiked with RIF and 25-DAR and solutions of standard RIF and 25-DAR in methanol over the range of 40–110 μg/ml (400–1100 ng/spot), is given in Table 1. In all the cases, linearity range was found to be 40–110 μg/ml for RIF and 25-DAR, with correlation coefficients of at least 0.99.

Precision of the method was determined by assessing intra-day and inter-day coefficients of variation for estimation of RIF and 25-DAR spiked in urine and the corresponding values varied from 2.03 to 3.31% and 3.45 to 6.15% for RIF and 0.69 to 1.90% and 3.76 to 12.01% for 25-DAR (Table 2). Reproducibility of sample application and measurement of peak area, in terms of R.S.D. (% C.V.) of peak area, was estimated and the R.S.D. values were found to be well below the specified limits.

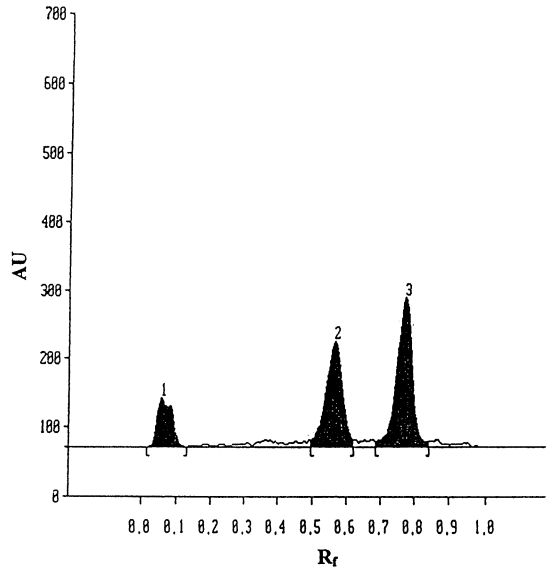

Fig. 1. Chromatogram showing separation of RIF (peak 3, $R_f = 0.74 \pm 0.02$) and 25-DAR (peak 2, $R_f = 0.55 \pm 0.02$) extracted from urine.

Table 1
Linear regression analysis of calibration curves for standard RIF and 25-DAR and RIF and 25-DAR spiked in urine

Sample	Equation	Correlation coefficient (r)
Std. RIF	$y = 8.667 \times x + 1542.710$	0.9943
Std. 25-DAR	$y = 9.569 \times x + 1.5268$	0.9985
Spiked RIF	$y = 8.690 \times x + 124.310$	0.9980
Spiked 25-DAR	$y = 7.692 \times x - 100.552$	0.9950

y = peak area for the peak of RIF or 25-DAR. x = concentration (μg/ml) of RIF or 25-DAR.

Table 2
Precision of proposed HPTLC method for estimation of RIF and 25-DAR spiked in urine

No.	Conc. (ng/spot)	Inter-day variation[a] (% C.V.)		Intra-day variation[b] (% C.V.)	
		RIF	25-DAR	RIF	25-DAR
1	400	3.45	12.01	3.31	1.90
2	600	5.20	5.95		
3	800	4.85	5.54	2.04	0.69
4	900	6.15	5.75		
5	1100	4.49	3.76	2.03	1.46

[a] Inter-day variation ($n = 5$).
[b] Intra-day variation ($n = 3$).

Accuracy of the measurement of RIF and 25-DAR in urine was determined by standard addition method at five different levels. It was found to be in the range of 97.02–102.20% and 97.41–105.40%, for RIF and 25-DAR, respectively (Table 3). The limit of detection and limit of quantification were found to be 7 μg/ml (70 ng/sot) and 40 μg/ml (400 ng/spot), respectively, for RIF and 25-DAR (Table 4). Specificity of the method was confirmed by the fact that endogenous urine components or related compounds (3-FRSV, $R_f = 0.67$; rifampicin quinone, $R_f = 0.89$) did not interfere in the separation and resolution of RIF and 25-DAR. Over and above, a high degree correlation between the spectra obtained at peak start, peak apex and peak end positions of individual spots of RIF ($r = 0.9997$) and 25-DAR ($r = 0.9999$) confirmed the purity of the corresponding spots (Fig. 2).

All the validation parameters for the proposed HPTLC method are summarized in Table 4.

Both the analytes, RIF and 25-DAR, were found stable over a period of 7 days at -20 °C.

Thus, the method was found to be simple, precise, accurate, sensitive and specific and can be employed for monitoring the urine levels of RIF and 25-DAR.

3.2. Comparative bioavailability study

3.2.1. Pharmacokinetic study

A typical chromatogram showing peaks for RIF and 25-DAR in urine samples of a volunteer collected at various time points after administration of RIF capsule is shown in Fig. 3. Using the proposed HPTLC method, amount of RIF and 25-DAR excreted over a period of 24 h after administration of RIF-INH FDC capsules and single component RIF capsules was determined. Comparative urinary excretion profiles (Cumulative mg excreted versus mid-point time (h)) of individual volunteers for RIF after administration of RIF-INH FDC and RIF alone capsules are represented in Fig. 4; while similar profiles for 25-DAR are shown in Fig. 5. From the average cumulative % excreted versus mid-point time plots for RIF and 25-DAR, it was observed that ∼13.05% (58 mg) of RIF and 13.8% (62 mg) of 25-DAR, with respect to dose, were excreted in 24 h after administration of RIF capsule formulation (450 mg) (Fig. 6(a–b)). On the other hand, ∼8.83% (39.7 mg) of RIF and 10% (45 mg) of 25-DAR, with respect to dose of RIF, were excreted, when RIF-INH FDC capsule formulation was administered (Fig. 6(a–b)). Thus, ∼120 mg (26.6% of RIF dose) and ∼84.7 mg (18.82% of RIF dose) of apparent RIF (total amount of RIF + 25-DAR excreted) was excreted after administration of RIF capsule and RIF-INH FDC capsule, respectively. This clearly indicates that there is ∼27.90% decrease in cumulative RIF excretion and about equivalent decrease (∼32.35%) in cumulative 25-DAR as well as apparent RIF excreted (29.1%) in urine, after administration of RIF-INH capsule as compared to RIF alone capsule.

Various pharmacokinetic parameters were determined from the plot of log (urinary excretion

Table 3
Accuracy data for proposed HPTLC method for estimation of RIF and 25-DAR spiked in urine

	Initial quantity spiked (ng/spot) (a)	Quantity of std. added (ng/spot) (b)	Total quantity spiked (ng/spot) (a)+(b)	Mean total quantity found[a] (ng/spot)	% Mean accuracy ($n=4$)
	400	0	400	388.08	97.02
	400	200	600	613.19	102.20
RIF	400	400	800	784.81	98.10
	400	500	900	875.20	97.20
	400	700	1100	1071.65	97.43
	400	0	400	389.66	97.41
	400	200	600	611.25	101.87
25-DAR	400	400	800	843.74	105.40
	400	500	900	879.30	97.70
	400	700	1100	1086.30	98.75

[a] Total quantity found was determined by utilizing corresponding calibration curve equations for RIF and 25-DAR spiked in urine for four replicate samples.

rate) versus mid-point time, i.e. log (dAU/dt) versus time, for both RIF and 25-DAR, after administration of RIF capsules as well as RIF-INH capsules. The mean log (dAU/dt) versus mid-point time (h) plots for RIF and 25-DAR are shown in Fig. 7(a–b), respectively. Various pharmacokinetic parameters include peak excretion rate ($(dAU/dt)_{max}$, mg/h), peak excretion time (t_{max}, h), area under excretion rate versus mid-point time curve up to 24 h (i.e. last mid-point 18 h), (AUC_{0-24}, mg) calculated by linear trapezoid rule, and area extrapolated to infinite time ($AUC_{0-\infty}$, mg) [$AUC_{0-\infty} = AUC_{0-24} + (dAU/dt)_{24}/k_{el}$; where $(dAU/dt)_{24}$ is the urinary excretion rate during last sampling interval], elimination rate constant (k_{el}, h^{-1}) determined from the slope of the terminal linear portion of log (urinary excretion rate) versus mid-point time plot (by using the formula $k_{el} = -2.303 \times$ slope) and elimination half-life ($t_{1/2}$, h), ($t_{1/2} = 0.693/k_{el}$). All these parameters are summarized in Table 5.

Average peak excretion rate for RIF after RIF capsule was 7.46 ± 1.81 mg/h and that after RIF-INH capsule was 5.88 ± 1.60 mg/h, which showed a reduction by $\sim 21\%$. Similarly, peak excretion rate of 25-DAR was reduced from 9.70 ± 2.60 to 7.37 ± 2.60 mg/h. It showed a corresponding decrease by $\sim 24\%$.

Calculated average peak time values for RIF after administration of RIF capsule and after RIF-INH capsule were 5.50 ± 1.73 and 7.33 ± 0.82 h, respectively, while, corresponding values for 25-DAR were 6.08 ± 1.49 and 7.66 ± 1.03 h (Table 5). These values indicated delay in the peak

Table 4
Summary of validation parameters for the proposed HPTLC method for estimation of RIF and 25-DAR in human urine

No.	Parameter	For RIF	For 25-DAR
1	Linearity range (ng/spot)	400–1100	400–1100
2	Precision (% C.V.)		
	Intra-day	2.03–3.31	0.69–1.90
	Inter-day	3.45–6.15	3.76–12.01
	Repeatability of measurement[a]	0.16 (<0.5%)	0.16 (<0.5%)
	Repeatability of sample application[b]	2.1 (<3%)	1.7 (<3%)
3	% Accuracy	97.02–102.20	97.41–105.40
4	Limit of detection (ng/spot)	70	70
5	Limit of quantification (ng/spot)	400	400
6	Specificity	Specific	Specific
7	Average extraction efficiency ($n=3$)	83.14%	81.08%

Note: [a,b]Figures in parentheses indicate the desired limits as per the instrument specifications.

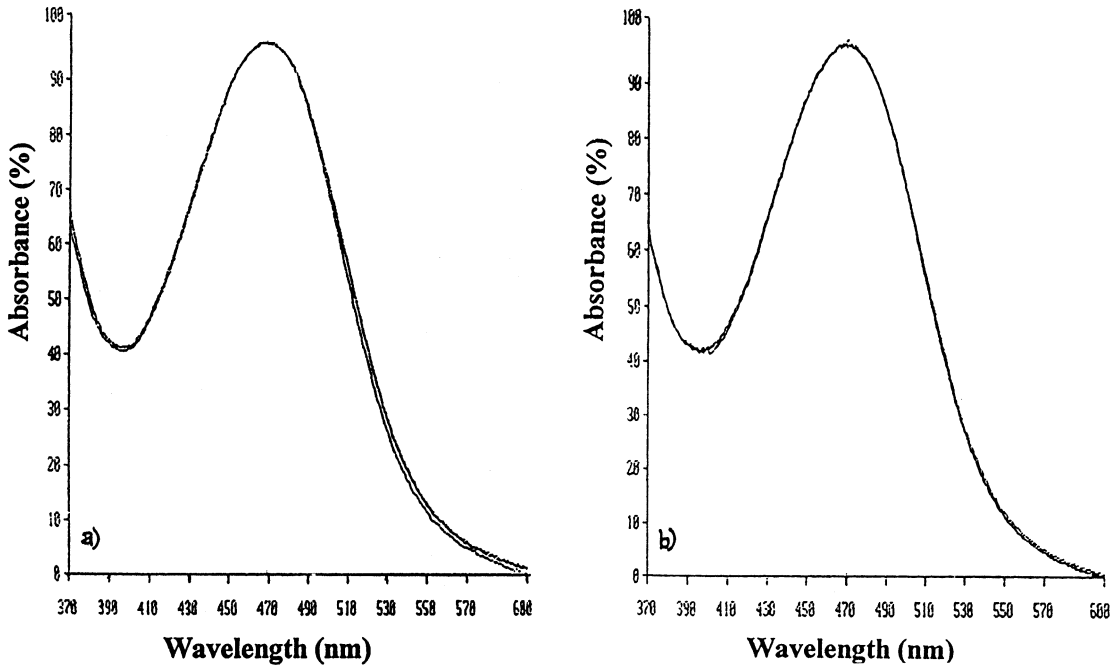

Fig. 2. Peak purity spectra for: (a) RIF; and (b) 25-DAR, extracted from urine sample, scanned at the peak start, peak apex and peak end positions of the corresponding spots (Correlation = 0.9997 and 0.9999, respectively).

excretion time when RIF was administered along with INH as compared to that of RIF alone.

Along with cumulative amount excreted, another measure of extent of absorption of RIF, AUC_{0-24}, for RIF and 25-DAR were calculated. The AUC_{0-24} values for RIF and 25-DAR after administration of RIF capsule and RIF-INH capsule, were 60.77 ± 16.01 and 39.96 ± 7.68 mg and 63.90 ± 12.03 and 45.20 ± 6.47 mg, respectively (Table 5). Thus, there was $\sim 34\%$ reduction in AUC_{0-24} for RIF and 29% in AUC_{0-24} for 25-DAR.

The average values of elimination rate constant for RIF after administration of RIF capsule and RIF-INH capsules were 0.292 and 0.302 h^{-1}, respectively and corresponding values for 25-DAR were 0.287 and 0.294 h^{-1}, respectively.

Similarly, mean elimination half life values for RIF and 25-DAR, after administration of RIF capsule and RIF-INH capsule, were 2.37 and 2.41, and 2.29 and 2.35 h, respectively.

3.2.2. Statistical analysis

It was observed that bioavailability of RIF from RIF-INH FDC capsule was significantly different as compared to that from RIF alone

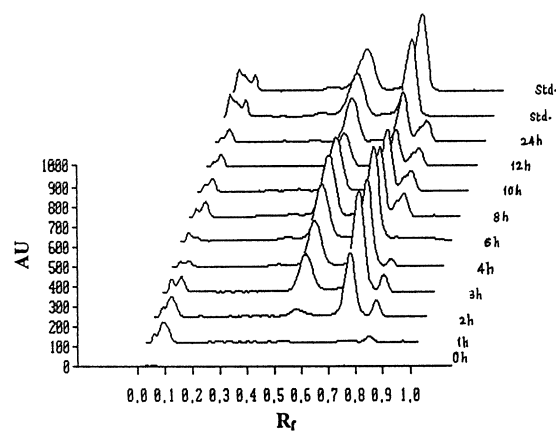

Fig. 3. Typical chromatogram showing levels of RIF and 25-DAR from the urine samples of a volunteer collected over a period of 24 h after administration of RIF alone capsule formulation.

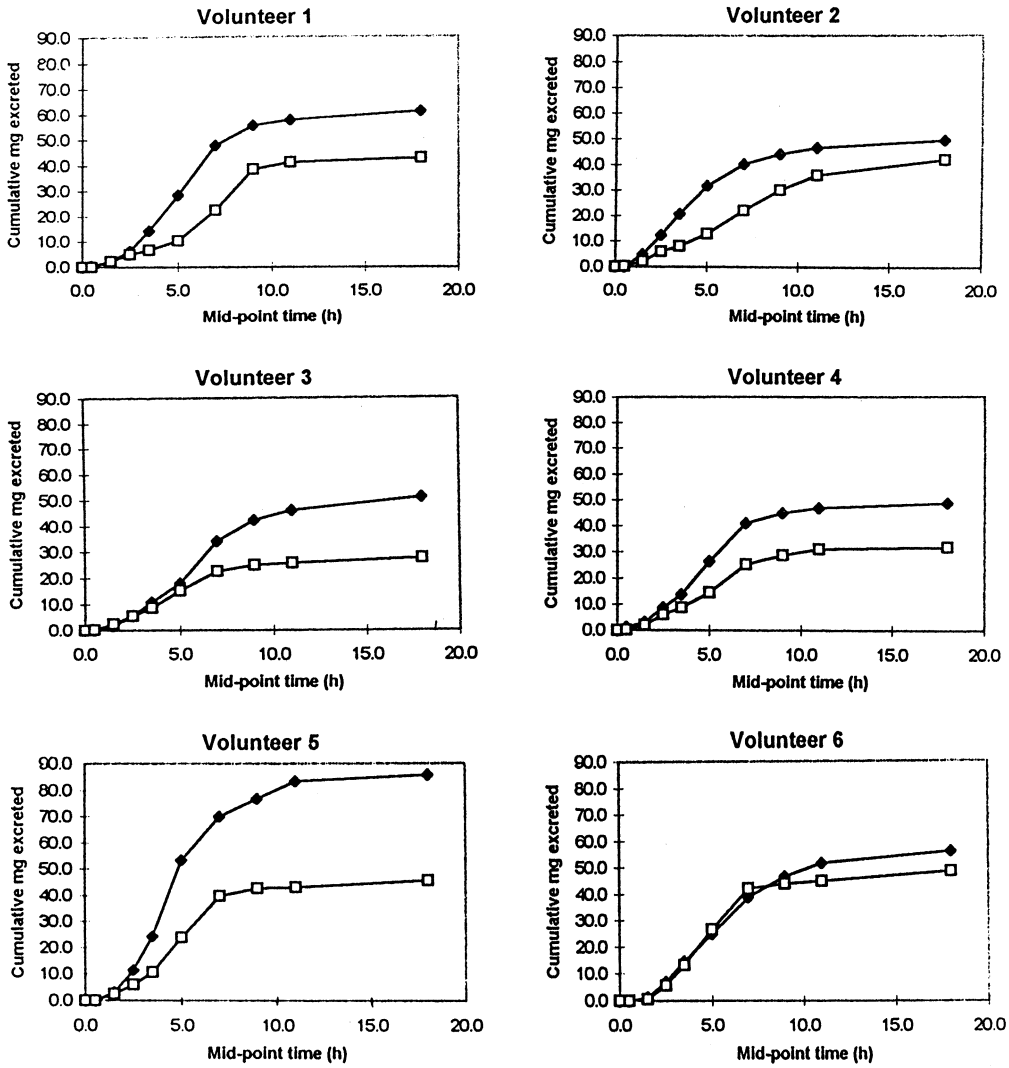

Fig. 4. Comparative urinary excretion profiles of RIF for individual volunteers (cumulative mg RIF excreted versus mid-point time plots) after administration of RIF alone (–◆–) and RIF-INH FDC (–□–) capsules.

capsule when various pharmacokinetic parameters were tested by ANOVA test. Calculated F values, obtained through ANOVA, for the bioavailability parameters: $(dAU/dt)_{max}$, AUC_{0-24} and cumulative amount of RIF/25-DAR excreted were found to be very high with respect to corresponding table values, indicating significant difference in the bioavailability of RIF after administration as FDC (RIF-INH) and that of RIF alone capsule (Table 6).

The currently accepted criteria by the US FDA for bioequivalency of most of the dosage forms requires the mean pharmacokinetic parameters of the test dosage form should be within 80 to 120% using untransformed data (or within 80 to 125% using log transformed data) of the reference dosage form using the 90% confidence interval (USP 24/NF 19, 2000). In the present study, 90% confidence intervals at lower level for the bioavailability parameters were invariably lower

than 20% limit as specified by the US FDA for the bioequivalence (using untransformed data) (Table 6). These results clearly show that there is a significant reduction in bioavailability of RIF from RIF-INH FDC capsule as compared to single component RIF capsule.

Relative Bioavailabilty of RIF, in terms of the ratio of average cumulative amount of RIF excreted after administration RIF-INH FDC to that of RIF alone capsules, demonstrated 32.35% decrease. Similarly, there was 27.90% decrease in the amount of 25-DAR excreted over a period of 24 h.

4. Discussion

Fixed dose combinations containing RIF along with INH, PZ and/or ETB are widely recommended for the treatment of tuberculosis, for better patient compliance and for avoiding devel-

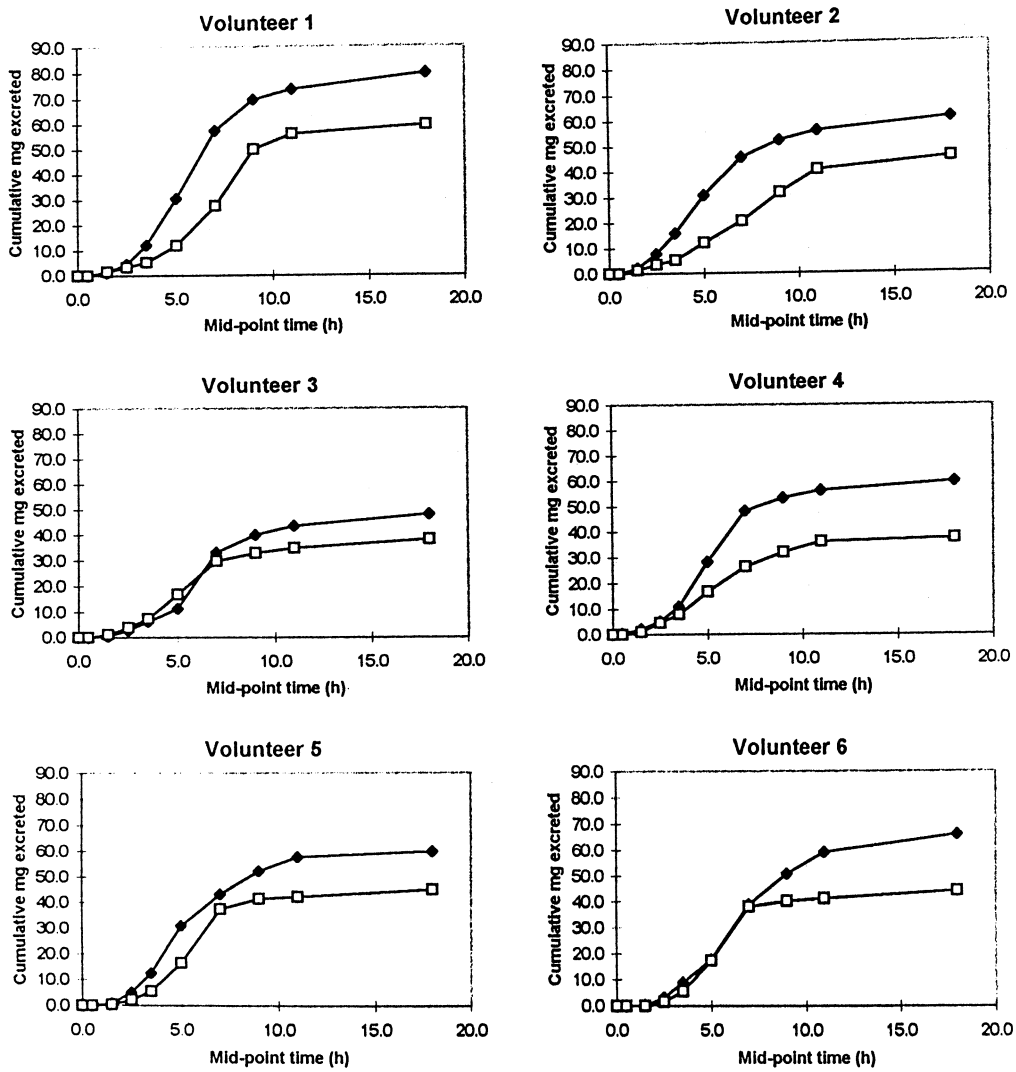

Fig. 5. Comparative urinary excretion profiles of 25-DAR for individual volunteers (cumulative mg 25-DAR excreted versus mid-point time plots) after administration of RIF alone (–◆–) and RIF-INH FDC (–□–) capsules.

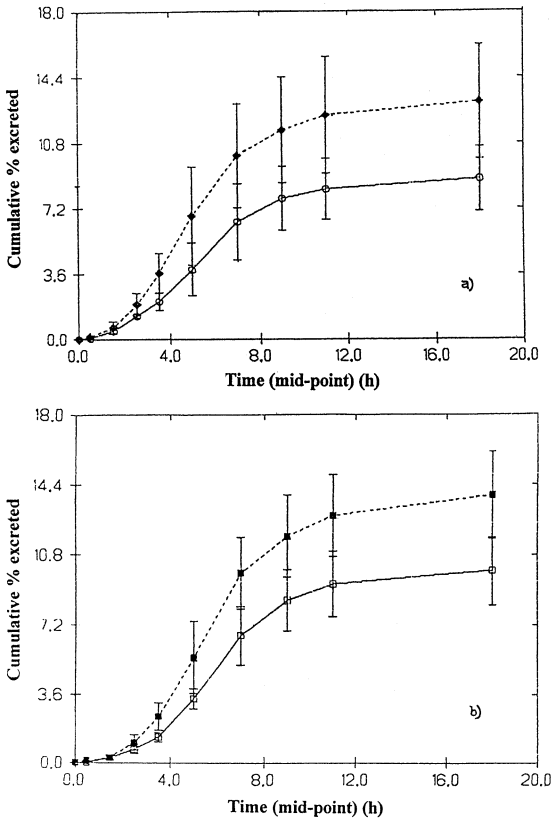

Fig. 6. Average cumulative % excreted, with respect to RIF dose, versus mid-point time plots for: (a) RIF; and (b) 25-DAR after administration of RIF alone (--■--) and RIF-INH FDC (--□--) capsule formulations to six healthy male volunteers (Note: The vertical lines indicate S.D. in cumulative % excreted at corresponding mid-point times).

opment of resistance to drug by *M. tuberculosis*. However, the problem of poor bioavailability of RIF from the FDCs containing INH has been reported and continues to be of much concern.

It was observed that in normal adults the peak plasma concentration (C_{max}) after administration of 600 mg RIF alone is in the range of 6–13 μg/ml and AUC_{0-8} in the range of 55–60 μg h/ml (Garnham et al., 1976; Man-Pyo-Chung et al., 1997; Peloquin et al., 1997; Ellard and Fourie, 1999); while on administration of RIF along with INH and/or PZ as separate formulations or as FDCs, the C_{max} values range from 3–6 μg/ml and AUC_{0-8} values in the range of 30–50 μg h/ml (Doshi et al., 1986; Ellard et al., 1986; Acocella, 1989; Zwolska et al., 1998; Padgaonkar et al., 1999; Revankar et al., 2000). Manufacturers in US observed 18% reduction in the bioavailability of RIF from the three drug FDC and to compensate for the reduced bioavailability, the dose of RIF was increased by 20% (Panel Discussion, 1999).

There are several contradictory reports suggesting that there is no statistical difference in the oral bioavailability of RIF after administration of RIF along with INH (Garnham et al., 1976; Ellard et al., 1986; Seth et al., 1993; Zofla et al., 1998; Zwolska et al., 1998; Gurumurthy et al., 1999; Padgaonkar et al., 1999; Panchagnula et al.,

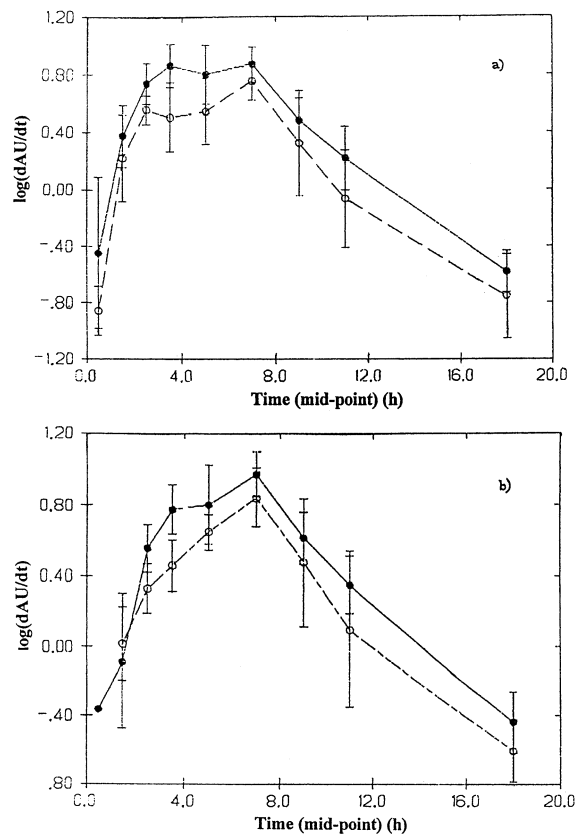

Fig. 7. Average log excretion rate (log (dAU/dt)) versus mid-point time plots for: (a) RIF; and (b) 25-DAR after administration of RIF alone (--●--) and RIF-INH FDC (--○--) capsule formulations to six healthy male volunteers (Note: The vertical lines indicate S.D. in log (dAU/dt) at corresponding mid-point times).

Table 5
Summary of pharmacokinetic parameters of RIF and 25-DAR after administration of RIF alone and RIF-INH FDC capsules

Pharmacokinetic parameter	RIF capsule		(RIF-INH) FDC capsule		% Decrease[a]	
	RIF	25-DAR	RIF	25-DAR	RIF	25-DAR
Cumulative amount excreted (mg)	58.73 ± 14.03	62.39 ± 10.25	39.72 ± 8.14	44.98 ± 7.86	32.35	27.90
Peak drug excretion rate (mg/h)	7.46 ± 1.81	9.70 ± 2.60	5.88 ± 1.60	7.37 ± 2.60	21.18	24.02
Time to peak drug excretion (h)	5.50 ± 1.73	6.08 ± 1.49	7.33 ± 0.82	7.66 ± 1.03	–	
AUC_{0-24} (mg)	60.77 ± 16.01	63.90 ± 12.03	39.96 ± 7.68	45.20 ± 6.47	34.24	29.26
$AUC_{0-\infty}$ (mg)	61.75 ± 15.85	65.29 ± 12.28	40.79 ± 8.07	46.13 ± 6.70	33.94	29.34
Elimination rate constant k_{el} (h^{-1})	0.292 ± 0.036	0.287 ± 0.028	0.302 ± 0.075	0.294 ± 0.041	–	
Elimination half life (mean) $t_{1/2}$ (h)	2.37	2.41	2.29	2.35	–	

Note: All the values indicate mean ± S.D. for the data from six volunteers.
[a] % Decrease in bioavailability parameter after administration of RIF-INH FDC capsule as compared to that obtained after RIF capsule.

1999a,b; Revankar et al., 2000) and have, thereby, added to the confusion. Most of these reports are, however, based on nonspecific microbiological methods.

Literature survey reveals that no systematic efforts have been made to compare the bioavailability of RIF from single component RIF formulations and combined RIF-INH FDC formulations.

Earlier we have reported that there is an increase in degradation of RIF released from RIF-INH FDC formulation as compared to single component RIF formulation in the in vitro dissolution study (Shishoo et al., 1999). Using specific analytical methods, we have demonstrated that INH catalyzes hydrolysis of RIF to poorly absorbed, insoluble 3-FRSV in acidic conditions. Evidence has been presented to indicate that RIF degradation is accelerated three folds in presence of INH due to reversible formation of insoluble hydrazone of 3-FRSV with INH (Singh et al., 2000).

The present work was undertaken to study the effect of INH on RIF bioavailability. A highly specific method (HPTLC) was employed to monitor RIF as well as its major active metabolite, 25-DAR, in urine. The comparative bioavailability study was carried out using RIF alone capsule and RIF-INH FDC capsule using urinary excretion data of RIF and 25-DAR.

Bioavailability of RIF was measured in terms of cumulative amount of RIF and 25-DAR excreted in 24 h, peak drug excretion rate $((dAU/dt)_{max})$ and AUC_{0-24} for RIF and 25-DAR from RIF-INH FDC and from formulation containing only RIF. A significant decrease was observed in all these pharmacokinetic parameters (Table 5). Cumulative amount of RIF and 25-DAR excreted in 24 h was reduced to the extent of 32.35 and 27.90%, respectively, after administration of RIF-INH FDC capsule as compared to RIF alone capsule. Peak excretion rate for RIF and 25-DAR showed a reduction of 21.18 and 24.02%, respectively, and corresponding decrease in AUC_{0-24} values was to the extent of 34.24 and 29.26%. These results clearly indicate that there is a considerable reduction in RIF bioavailability when it is administered along with INH in a FDC formulation, which correlates well with our observations from the earlier in vitro studies (Shishoo et al., 1999).

The data obtained from the present comparative bioavailability study lends support to our earlier apprehensions of impaired bioavailability of RIF in presence of INH.

Recent Model Protocol issued by WHO for establishing bioequivalence of RIF in FDCs (Fourie et al., 1999) and most of the bioequivalence studies for RIF from two or three drug FDCs consider comparison of various pharmacokinetic parameters of RIF obtained after administration of FDC and administration of separate formulations of individual components (loose combination) administered at the same

time (Garnham et al., 1976; Ellard et al., 1986; Seth et al., 1993; Zofla et al., 1998; Zwolska et al., 1998; Gurumurthy et al., 1999; Padgaonkar et al., 1999; Panchagnula et al., 1999b; Revankar et al., 2000). However, both FDC and the separate formulations will face the similar acidic pH of stomach and RIF will suffer the same fate in both the cases (i. e. It will degrade to the same extent in presence of INH). Hence, no significant difference is observed in the bioavailability of RIF form various FDCs tested. But, in absolute terms, the bioavailability of RIF will be impaired as compared to RIF alone formulation.

Further, it is recommended that RIF oral dosage form should be taken on empty stomach or at least 1 h before or 2 h after meals (USP DI, 1996). But, the pH of gastric fluid at this stage remains more acidic (pH 1.4–2.1) as compared to stomach after meals (pH 3.4–5.4) (Kararly, 1995). Therefore, there is a likelihood of higher decomposition of RIF after administration of FDC formulation on an empty stomach. The acceleration of RIF degradation process due to presence of INH will further reduce the amount of RIF available for absorption. This may lead to poor bioavailability of RIF from FDC.

The results obtained from the present in vivo study, indicate significant reduction in bioavailability of RIF from FDC as compared to that of RIF alone. This could be due to the enhanced degradation of RIF in stomach in presence of INH from the RIF-INH FDC formulation.

Thus, there is an urgent need to modify the FDC formulation in such a way that RIF and INH are not released simultaneously in the stomach or both the drugs need to be administered separately after an interval corresponding to average gastric residence time.

5. Conclusions

Bioavailability of RIF is significantly impaired when it is administered along with INH as a FDC, in comparison with administration of formulation containing only RIF. This lends confirmation to earlier in vitro studies indicating INH accelerates degradation of RIF into its insoluble, poorly absorbed derivative-3-FRSV in acidic environment of stomach via reversible formation of

Table 6
Statistical comparison of different pharmacokinetic parameters of RIF and 25-DAR obtained after administration of RIF-INH FDC capsule and RIF capsule

	RIF		25-DAR	
ANOVA	F_{cal}	F_{table}	F_{cal}	F_{table}
Peak drug excretion rate	10.54	7.71	4.233	7.71
AUC_{0-24}	21.09	7.71	20.93	7.71
Cum. amount excreted	26.88	7.71	84.72	7.71
90% C.I. ratio[a]	Lower limit (80%)	Upper limit (120%)	Lower limit (80%)	Upper limit (120%)
Peak drug excretion rate	52.34	105.29	52.28	99.59
AUC_{0-24}	49.87	81.65	57.10	84.37
Cum. amount excreted	54.33	80.93	65.64	78.55
Relative bioavailability[b] (Cum. amount excreted)	67.63%		72.09%	

F_{cal} and F_{table} = calculated and table value of 'F' statistics.

[a] 90% confidence interval for the ratio of corresponding pharmacokinetic parameters (ratio of parameters for RIF after administration of RIF-INH FDC capsule to the parameters after capsule containing only RIF).

[b] (Ratio of cum. RIF or 25-DAR excreted in urine in 24 h after administration of RIF-INH FDC capsule to RIF or 25-DAR excreted after RIF capsule) × 100.

hydrazone of 3-FRSV with INH. There exists a qualitative correlation between acceleration of in vitro degradation of RIF in presence of INH in acidic medium and decrease in the in vivo bioavailability of RIF when administered as combination formulation with INH (RIF-INH). The present study underlines the fact that reduced bioavailability of RIF from RIF-INH FDC formulations may be one of the factors responsible for development of resistance to RIF.Thus, there is a need to develop stable formulations containing RIF-INH combination to withstand the acidic environment of stomach, like enteric coated tablets or alternative multilayered formulations (other than conventional FDCs containing RIF along with other antitubercular drugs) such that INH is first released in stomach and RIF is released in upper part of intestine. Over and above, administration of FDCs with two/three/four drugs on an empty stomach produces severe gastric irritation and hence the patient non-compliance to the formulation. This tempts one to reconsider the advice to administer RIF formulations (single component or FDC) on empty stomach and whether it can be administered after meals when the pH of stomach is less acidic.

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