Treatment of tuberculosis: challenging the World Health Organization recommendation of simultaneous oral administration at the same time of the day of rifampicin, isoniazid and pyrazinamide

Alessandro Freddi
Independent consultant
P. O. Box 8973 Asmara Eritrea – tel 00393387231051 – sandrofreddi@gmail.com
Keywords: FDC, IUATLD, hydrazine, ethambutol, levofloxacin

Summary: Mortality for tuberculosis is very high in low-income countries. The World Health Organization and the International Union against Tuberculosis and Lung Disease recommend administrating the anti-tuberculosis drugs together at the same time of the day, an order not an advice for most of these countries. Critical examination of relevant literature reveals that level of rifampicin bioavailability in the administration of the quadruple association with isoniazid, pyrazinamide and ethambutol at the same time of the day is sub-therapeutic and that the corresponding information concerning the triple association without ethambutol is poor and questionable. Rifampicin proper absorption requires the characteristic acidity of the empty stomach. Here it is proposed that a particular chemical reaction occurs in the stomach at such pH between rifampicin and isoniazid, catalyzed by pyrazinamide, which produces the highly hepato-toxic substance hydrazine. The role of rifampicin is essential in the short-course treatment, therefore, tuberculosis death toll could simply be the consequence of the use of triple and quadruple fixed-drug combination pills. The communication also comprises an attempt of comparison of rifampicin bioavailability among different studies and a proposal of explanation of the loss of the drug bioavailability in consequence of improper stomach acidity.

1 – INTRODUCTION
In low-income countries, tuberculosis (TB) is a widespread disease, for instance in Ethiopia in one year in the period 2001-2002 about one hundred thousand new cases have been notified, among a population of about 67 million inhabitants. In some areas of Cape Town in South Africa the incidence of TB can exceed 1% per annum. The rate of mortality for TB reaches 20% in these countries. For instance in Eritrea 43 of the 245 patients, who had started treatment with anti-TB drugs in the year 2004 in 2 out of the 6 public health centres of Asmara, died during the period of the treatment (author’s examination of Edaga Hamus Health Center and Semenawi Health Center official registers).

Old treatments, which were based on streptomycin (S) and isoniazid (H), lasted 1.5-2 years. The most common were 2SHP/16-22HP and 2SHE/16-22HE (P = PAS, E = ethambutol, figures preceding symbols of the drugs
represent the number of months of the respective phase of treatment). Starting from the end of the sixties of the
last century these treatments were replaced by others containing rifampicin, named short-course, as the actual
protocols 2HZER/4HR and 2HZER/6HE (Z = pyrazinamide, R = rifampicin).

The World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease
(IUATLD) recommend administrating the anti-mycobacterial drugs for tuberculosis together at the same time of
the day. The health authorities of the low-income countries are under the pressure from the WHO both to follow
this advice and to refrain from using rifampicin in the continuation phase of the treatment, the latter whenever the
patient is older than 7 years, or 14 years if the disease is severe. The poorer the country, the larger the pressure,
that lies in supplying a great amount of fixed-drug combination (FDC) pills containing HZER and HE to these
countries for free, but not rifampicin single-drug pills. Therefore, in case of separate administration of the drugs at
different times of the day, a country would be forced purchasing them and would even risk losing other grants for
different health need, which are usually supplied by the WHO to the these countries.

In the clinical practice, the simultaneous ingestion of HZR and HZER usually occurs after meal because of gastric
irritation. In fact it has been reported that, if “the patient wants to take the FDC of three or four drugs on an empty
stomach, the gastric irritation is so severe that there are times that compliance will be very poor.” (Bakhle, Lupin)

“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.” However, the bioavailability of rifampicin is reduced if the
drug is ingested after meal. In July 1976, short-term chemotherapy for tuberculosis was introduced in Chingola,
Zambia, based on daily rifampicin and isoniazid for 9 months. This is excerpt of the experience report- “New
cases are treated as inpatients until they are sputum-negative and showing clinical and radiological improvement
(on these criteria most patients are now discharged within 4 weeks, showing a degree of improvement which
would have taken several months to achieve on our previous streptomycin-based therapy). However, it soon
became apparent that a small number of patients were showing no such improvement, even at 4 weeks, and
inquiry into ward routine revealed the cause: the drugs were being given at 9 a.m. after breakfast. Since July, 48
new patients with tuberculosis have been started on the rifampicin-based regimen, and 8 (17%) have shown this
failure to respond. Each of these was merely treated by giving rifampicin at the previous drug round (3 a.m., 7 h
after the last meal) and all showed a rapid response within 2 weeks. We now give rifampicin routinely at 3 a.m.,
and since this modification was introduced all patients have responded well.” The effect of food on rifampicin
bioavailability was already known, results “are perfectly clear: in the case of Rimactane, it is obvious that the
ingestion of food does adversely influence the quantity and speed of absorption”, fig 1. This is confirmed also by
other studies. The decrease of absorption can arrive, depending on quantity and type of ingested food, up to
over 50% of the dose.

Rifampicin had been invented before 1966 at the Lepetit laboratories. The firm realized the clinical
experimentation of the drug with Ciba support, which got licence to produce the drug. Lepetit marketed rifampicin
with the name Rifadin, Ciba with the name Rimactane. Later Lepetit became property successively of Hoechst, Marion-Russell, Merrell-Dow, Aventis and Sanofi. Scientific research at Lepetit was carried out in the installation of Gerenzano (Italy). Hoechst ceded this installation to the researchers working there. The new co-operative society, named Biosearch, then merged with a small US firm, Vaicuron (49% Biosearch 51% Vaicuron) and the resulting society was later purchased by Pfizer, which sold Lepetit collection of microbial strains to a consortium of pharmaceutical corporations and ceded the rest of the installation to a group of Italian and Swiss public institutions.

The present communication provides information supporting that the simultaneous administration of HZR at the same time of the day makes always rifampicin bioavailability scarce, not only in case of presence of food in the stomach, and proposes a theoretical explanation.

2 – ADMINISTRATION OF HZR TOGETHER AT THE SAME TIME OF THE DAY- PHARMACOKYNETIC STUDIES

Simultaneous administration of HZER pills at the same time of the day caused 39% reduction of rifampicin serum AUC₀→₁₂ (area under concentration curve) in comparison with the drug taken alone. In another study, which had been realized in collaboration with Lepetit on 12 patients, no reduction of bioavailability instead resulted from the association of HZR at the same time of the day, both in free and in fixed combination, fig 2, tab 1 and 2. However, this result should be considered with suspicion, because the reference single-drug rifampicin had been administered to only four patients and the relative curve of plasma concentration rises after the 8th hour, suggesting difficulties in the drug intestinal absorption, fig 2 and tab 2. For instance dummy pills, made of a high specific surface insoluble powder, which adsorbs rifampicin reducing its bioavailability, might have been given to the four patients, since no declaration of awareness of the 12 patients concerning the kind of experiment had been reported.

One of the authors of the questioned study, Acocella G, later joined the Reference Centre for Chemotherapy of Mycobacterial Diseases of the University of Pavia, where he realized “carefully planned and executed studies on the bioavailability of different brands of rifampicin alone, double combinations of rifampicin and isoniazid and formulations of rifampicin, isoniazid and pyrazinamide, which had been produced by different firms in the world. "All four brands of triple combinations tested had the same drug content: isoniazid 100 mg, rifampicin 150 mg, pyrazinamide 500 mg." All the four were associated with reduced bioavailability of rifampicin. Results of this study have been published, with few details, in the proceeding of a satellite symposium, which took place during the Annual Meeting of the IUATLD in Dubrovnik in 1988, and had already been presented in 1987 at the Chemotherapy Experts Meeting of the IUATLD in Paris, according to citation in a more recent meeting of the same Organization. In the experiment on the triple FDCs only isoniazid had been indicated as companion drug of rifampicin in the reference association of the drugs taken at the same time of the day in separate pills (free
combination), not pyrazinamide. So, the observed reduction of rifampicin bioavailability from all the triple FDCs could have been the consequence of adding pyrazinamide to the bolus, thus indicating that the simultaneous presence of HZR in the stomach reduces rifampicin bioavailability. On the other hand, it is possible, that pyrazinamide was a component of the reference association and this piece of information had not been considered important by the author. However, the former is more probable, since a deliberate shadowing of the issue relative to the absence of pyrazinamide could explain why the study has not been published in a peer-reviewed journal. Reichman L said about this study- "I recall in the 1980s that Gianni Acocella was not allowed by his Dean to publish his data in a peer-reviewed publication...". 7

Except these three studies, the bioavailability of rifampicin in the case of HZR associations seems described in the literature solely comparing FDC pills with the free combinations of the same drugs, i.e. with the same drugs in different pills taken at the same time of the day. Obviously, that excludes the possibility of disclosing any reduction of bioavailability, which is caused by the simultaneous presence of the drugs in the stomach, as it has been already pointed out: 8,24

Anyway, at least in the HZER association, the loss of bioavailability is confirmed also by another kind of evidence. Rifampicin bioavailability is lowered by 20% or more adding anti-acids to the single-drug, 18,25 because the characteristic strong acid environment of the empty stomach (pH ≤2) is necessary for the good absorption of the drug, as confirmed by the experience with gastro-resistant pills, since blood "levels obtained with coated tablets of rifampicin are significantly lower. (Personal communication of Dr. J. Büttner, Ciba AG, Basel)." 26 However, no decrease of the drug bioavailability is revealed if aluminium hydroxide and magnesium hydroxide are added to the association of HZER pills at the same time of the day. 27 It follows that, in the experiment, a quantitatively similar loss of the drug bioavailability has been induced also by the simultaneous presence of HZER in the stomach or by excipients in their pills. It must be underlined also that such loss did not add together the one which is induced by anti-acids. Therefore, either its causative phenomenon acted in alternative to the phenomenon, which is responsible in the case of anti-acids, or the same phenomenon is responsible in both cases.

The reduced bioavailability of rifampicin, which is induced by the simultaneous administration at the same time of the day of HZER active principles (and probably also HZR) seems the consequence of the following process.

3 – CHEMICAL REACTION AMONG HZR IN THE STOMACH

Rifampicin, which is a hydrazone, is subjected to the equilibrium of azomethynic bond hydrolysis in acid environment with formation of 3-formylrifamycin SV (3FRSV) and 1-amino-4-methylpiperazine, 28 fig 3a. The rate of hydrolysis is the least at pH =5 and increases with increasing difference from this value of pH. 26,29 The consequent decomposition of rifampicin has been considered significant in relation to the drug residence time in the empty stomach. The half-life of the reaction at 37°C in 0.1M aqueous HCl is 5.9 h and 1.08 h for initial concentrations of the drug 1 mg/mL and 20 mg/L respectively. 26 The general kinetic expression of hydrolysis of
aldehydic imino-derivatives is rather complex but, for the purpose of the present communication, it can be summarized in the following equation, which is applied to the case of rifampicin, where the constants of velocity $\kappa_{H1}$ and $\kappa_{H2}$, positive quantities, are a function of pH:

$$\text{vel.} = \kappa_{H1}[R] - \kappa_{H2}[3\text{FRSV}][1\text{-amino-4-methylpiperazine}].$$

Isoniazid could react with 1-amino-4-methylpiperazine to produce hydrazine and N-(4-methyl-1-piperazinyl)isonicotinamide, fig 3b, according to the mechanism reported in fig 3c. This reaction consumes 1-amino-4-methylpiperazine increasing the hydrolysis rate of the azomethynic bond of rifampicin, according to the kinetic expression above. In fact it has been shown that in 0.1M HCl at 50°C “the rate of rifampicin degradation can be decreased by addition of 1-methyl-4-amino-piperazine to the reaction solution.”

The proposed mechanism implies consumption of rifampicin and isoniazid, catalysis by pyrazinamide and production of hydrazine, which is replaced by 1-amino-4-methylpiperazine in the bond with the isonicotinoyl residue of isoniazid.

It has been said about the HZR association (Ellard G): “There is no possible chemical reaction between the three drugs.” However, authors measured hydrazine concentration in the blood of patients who received HZR and, having found no correlation between hydrazine plasma levels and the patient velocity in acetylating isoniazid, they concluded that hydrazine forms during the first hour after the administration of the drugs. Hydrazine is highly hepato-toxic.

### 4 – MISSED SOLUBILIZATION OF RIFAMPICIN IN THE STOMACH

In the case of rifampicin ingestion with anti-acids, the loss of bioavailability could be due to an incomplete solubilization of the drug. The following interpretation accounts for the relation between pH ≤2 in the stomach and proper gastrointestinal absorption of the drug.

Rifampicin solubility in aqueous environment is 1 mg/mL at pH 3, which is 100 times less than at pH 2, tab 3. In practice, the drug has required an even greater proportion of acidulated water to completely solve at pH ≥3, 2.5 milliliters for each milligram of rifampicin. A lot of specimens of the drug, either in pure form or in commercial bulk formulations, which have been tested for solubilization at pH ≥3, have solved by less than 50% after 1 hour, despite, for each milligram of rifampicin, 1.7 mL of solvent were used in one case and 6 mL in another. It should be expected that the remaining percentage would have taken a couple of months to solve. Stability of rifampicin in water decreases also with increasing dilution, a pattern that becomes exponential for high dilution, fig 4, in consequence of the very low solubility of 3FRSV, which is about 6 mg/L. In fact the concentration of 3FRSV is constant in the comparatively more concentrated solutions because it is limited by the substance solubility. Any difference of solution volume, i.e. of concentration of the other chemical species in the kinetic expression reported in the previous chapter, affects about equally the rate of both direct and reverse microscopic reaction processes. On the other hand, whenever the dilution is so high that 3FRSV concentration becomes lower
than its solubility, the reverse microscopic reaction process becomes of the 2nd order and any further increase of

dilution decreases the rate of the reverse microscopic reaction much more than the rate of the direct microscopic
reaction, strongly increasing the hydrolysis rate. Therefore, at pH ≥3 the scarce solubility of rifampicin causes
significant production of 3FRSV despite pH is not particularly high or low.

The structures of rifampicin and 3FRSV are similar and precipitation of 3FRSV on the surface of the crystals of
rifampicin could be the reason of the interruption of the drug solubilization at pH ≥3, an event that does not occur
at pH ≈2 and below. For such pH values, indeed, an uninterrupted flux of rifampicin molecules leaves the crystal
surface because of its high solubility.

Several drugs and excipients contribute to neutralize the gastric juice and to buffer the stomach at pH ≥3 but an
analysis concerning their identity and the respective amounts, for which the effect should be regarded as
significant, is outside the purpose of the present communication. It is possible that the reduction of rifampicin
bioavailability in the above considered two pharmacokinetic studies with HZER (ref 16 and 27) had been caused
by an anti-acid effect of the excipients of the pills, not by the hydrazine-producing reaction, which has been
proposed in the previous chapter. Since these are the sole two studies confirming a loss of rifampicin
bioavailability in case of simultaneous presence of HZR in the stomach, the hypothesis of the reaction in the
stomach could appear scarcely supported. However, if the occurrence of a rifampicin-decomposing reaction in the
stomach at pH ≈2 were not real, it is quite unlikely that producers would not have chosen to attain such pH, by
means for instance of a proper choice of excipients. Thus, it would be quite difficult to explain the lack of a proper
pharmacokinetic demonstration of good rifampicin bioavailability in the conditions of the title. Therefore, the
hydrazine-producing reaction should be suspected to significantly occur in the stomach, at least whenever the
mixture of HZR are present together in the stomach at pH ≈2 and below. Results are reduction of rifampicin
bioavailability and hydrazine poisoning of the patient.

As about the ingestion of rifampicin as a single drug after meal, the loss of bioavailability is probably due to the
reduction of the drug flux in the portal blood. It implies that a greater amount of the drug is metabolized in the first
pass in the liver.

Obviously, a reduction of rifampicin bioavailability can result also from a wrong production of the single-drug
pill, but it can be easily prevented.

5 – OTHER STUDIES CONCERNING RIFAMPICIN BIOAVAILABILITY

The anti-tubercular FDC pills used nowadays in the world are manufactured by many firms from different
countries. The first triple FDCs have been prepared and marketed by Lepetit. They were Rifater 5 (H 75mg, Z
400mg, R 150mg), also named Rifater 75, Rifater 2, also named Rifater 50, and Rifater 3, the last one for the
intermittent treatment, tab 1. Rifater 5 was tested in a clinical trial in the US in 1984, shortly after it was marketed in the country. In 1988 it was already on the market of other countries (Ziersky, RFA).

It has been stated, addressing Rifater 2 (Frieden T, WHO and US CDC)- “We know that the manufacturer that licensed the three-drug FDC based in the US increased the dose of rifampicin by 20% because in their volunteers the absorption of rifampicin was reduced by 18% and that is with a leading manufacturer using the best available mechanisms….” Roscigno G (Hoechst Marion Russell) commented- “You said that the only manufacturer that registered Rifater in the US had to increase the rifampicin. This is absolutely false. We submitted the registration of Rifater so, which is RMP 120, INH 50 and PZA 300, to the FDA in 1994 and got approval in 1996, based on two clinical trials done in Africa—one in Zaire, as a matter of fact, and the other by Professor Chaulet in Algeria. There was no issue at all from the FDA that requested us to increase the dosage of rifampicin. So this information is not correct. It was submitted as 120, 50 and 300, and in the market today it is 120, 50 and 300”. Frieden added- “I don’t want to get into a dialogue, but I just stand by 100% what I said, that the testing in US showed 18% reduction bioavailability, and for that reason FDA did not ask HMR (MMD at this point) to increase, but they did. If you take 120, 50 and 300 and take the usual multiplier of six, that gives 720 mg of rifampicin. That’s why it was done. I don’t want to enter into a debate here.”

It is difficult to compare rifampicin bioavailability among different studies because of different reasons. Rifampicin concentration in the blood decreases during about the first 10 days of administration, refs 37,38,39 in tab 1. The phenomenon is known as liver metabolic autoinduction. Also the half-life of the drug decreases during the same period. Disparity of rifampicin bioavailability between sexes seems greater than expected on the grounds of simple body mass disparity, ref 40 in tab 1, a phenomenon which has been poorly studied. Area under curve of concentration (AUC_{0→∞}) of rifampicin in the blood increases with the square of the oral dose, fig 5, but the relative experiments did not report the mass of subjects, despite perhaps something has been written about the point. This obstructs normalization of the drug blood concentration data, which is necessary to compare results, which have been obtained from different doses per kilo of body weight (b.w.). The phenomenon has been interpreted as the consequence of saturation of the biologic system for biliary excretion of the drug.

Acocella and co-workers realized at the Centre of Pavia an uncompleted tetralogy of studies, tab 1, in which plasma concentrations of Rifater 2 and Rifater 3 had been measured in different conditions, Rifater 2 with relatively low dosage in a single-dose study and with a relatively high dosage during repeated administrations, Rifater 3 only with a relatively low dosage in a single-dose study. As about the missing study of Rifater 3, it has been stated- “Studies are underway in our centre to evaluate the time course of the plasma concentrations of isoniazid, rifampicin and pyrazinamide in fixed combination for intermittent use on repeated (three weekly) administration to assess, in particular, the existence of possible cumulation phenomena for pyrazinamide.” However, Gianni Acocella prematurely died and results of this study have not been published.
Pharmacokinetics of Rifater 2 and Rifater 3 had been already studied in Singapore and Hong Kong. As for Rifater 2, “it was shown that the plasma concentrations of isoniazid were remarkably lower than those generally found after a dose of 250 mg of the drug (five tablets of the new formulation).” The AUCs$_{0-\infty}$ of isoniazid plasma concentration were 9 and 23 mg h/l in 6 fast and 2 slow isoniazid-acetylators, respectively, who were being taking 4.9 mg/kg b.w. daily of the drug. As about rifampicin, the authors commented their results—“Although the areas under the plasma concentration/time curves found after giving 600 mg doses of rifampicin were only about 70% of those reported by Buniva and co-workers the discrepancy is almost certainly because the present study was carried out in rifampin-induced patients rather than in uninduced volunteers.” In the cited article by Buniva et al, results from 14 studies had been reported, which had been realized at Lepetit, but 1, the numbers of subjects, who participated in each of these studies, had not been indicated. The average value, which is possible to calculate on such limited base, shows that, in comparison, the AUCs in the Asian study are 70% in the case of Rifater 3, but only 56% in the case of Rifater 2. Moreover, weight of the patients in Asia was 51 kg and Ellard at al omitted to mention that Lepetit volunteers average weight was 71 kg. Surprisingly, Fox W commented the study, stating that the measures “indicated the excellent bioavailability of each of the three drugs in the two combined formulations.” It must also be pointed out that the Asian study contains approximations, does not indicate the sex of patients and that the original data “had long since been discarded.”

6 – EXPECTED CLINICAL CONSEQUENCIES FROM THE LOW BIOAVAILABILITY OF RIFAMPICIN

The WHO recommends adult TB patients to take about 10 mg/kg once daily up to 600 mg daily. Results of a clinical trial in the USA with 5HR/7-18HE suggest “RIF dosage of less than 9 mg per kg of body weight per day may be inadequate for treatment of pulmonary tuberculosis.” Serial counts of viable tubercle bacilli in the sputum, which had been recorded in the first days of unusual drugs treatments, suggest that rifampicin minimal bactericidal concentration in vivo sharply decreases with the dose and, therefore, “the usual dose of about 10 mg/kg rifampicin appears to be only just sufficient”. “In vitro studies on the bactericidal activity of rifampicin have indicated that the Minimal Bactericidal Concentration (MBC) of the antibiotic against Mycobacterium tuberculosis is of the order of 1 mg/L in Tween-containing medium. The value of the MBC must be increased by a factor of 10 to 15 if the MBC is assessed in a medium not containing Tween, a condition similar to that existing in the infected area of the human lung.” Therefore, the reduced bioavailability of rifampicin, which has been observed taking HZER together at the same time of the day and which is probably always associated with the simultaneous presence of HZR in the stomach, should be considered sub-therapeutic.

The letter from Zambia in chapter 1 and other clinical data demonstrate that rifampicin heals TB in much shorter time than the drugs of previous regimens. Rifampicin is just the drug, which has allowed the shortening of TB treatment from the original 1.5-2 years to the present 6-8 months. Pyrazinamide contributes to the acceleration of the healing process too, but less than rifampicin. Therefore, it should be expected that the scarce bioavailability
of rifampicin in the initial phase of the treatment is the main reason of the disease relapse rate and the main reason, whenever the disease is primary H-resistant, of the treatment failure. This implies development of the double resistance (HE or HR, according to the continuation phase employed), since pyrazinamide is ineffective or scarcely effective at preventing the emergence of resistance in the companion drugs. Pyrazinamide is active only at pH <5.6 and it is highly probable that large numbers of bacilli live in less acidic environment in the patient.

In conclusion, recommendation by the WHO and the IUATLD of simultaneous oral administration of rifampicin, isoniazid and pyrazinamide at the same time of the day is not rational.

Acknowledgments
The author thanks Stefano Falteri, Doctor Christian Fiala, Professors Andrew Henxheimer, Pieralfredo Mancini and Luigi Mandolini.

Conflict of Interest
None to declare.

References


Table 1 - Serum/plasma concentration of rifampicin in different studies.

<table>
<thead>
<tr>
<th>formulation under study</th>
<th>subjects (quantity and sex)</th>
<th>dose (mg)</th>
<th>average body mass (kg)</th>
<th>relative dose (mg/kg b.w.)</th>
<th>day of the treatment</th>
<th>C (mg/l)</th>
<th>AUC (mg h/l)</th>
<th>C_{AUC} (mg/l)</th>
<th>C_{AUC} (mg/l)</th>
<th>reference, year of publication</th>
<th>method of measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 26 F 42 M</td>
<td>600 57.2 10.5 2°</td>
<td></td>
<td>11.3 t_h 22.5 t_h 16.2 t_h 7.9 t_h 3.0 t_h</td>
<td>1°</td>
<td>2.0 1.7 1.4 1.1 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20.8 11.7 12.9 7.2 4.5</td>
<td>Bacillus brevis</td>
</tr>
<tr>
<td>R capsule 6 1°</td>
<td>600 67.6 9.2</td>
<td></td>
<td>6.3 max 3.2 36.0 5.6 4.1°</td>
<td>1°</td>
<td>2.0 1.7 1.4 1.1 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>free combination of HZR</td>
<td>600 81.3 7.4</td>
<td></td>
<td>9.9 max 2.0 5.0 7.6° 4.1°</td>
<td>1°</td>
<td>2.0 1.7 1.4 1.1 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 tab 2 fig 2 1985 13 1983</td>
<td>Sarcina lutea</td>
</tr>
<tr>
<td>experimental FDC 4M</td>
<td>600 73.5 9.2</td>
<td></td>
<td>10.8 max 1.7 6.0 10.1° 5.8°</td>
<td>1°</td>
<td>2.0 1.7 1.4 1.1 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>Sarcina lutea</td>
</tr>
<tr>
<td>Rifater 2 8 600 51 11.8</td>
<td></td>
<td></td>
<td>1° 10.6 max 2.1 7.3 8.7 5.4</td>
<td>46 1988</td>
<td>45 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>HPLC</td>
</tr>
<tr>
<td>Rifater 3 600 51 11.8</td>
<td></td>
<td></td>
<td>1° 10.6 max 2.1 7.3 8.7 5.4</td>
<td>46 1988</td>
<td>45 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>HPLC</td>
</tr>
<tr>
<td>Rifater 2 2F 8M 600 67.6</td>
<td></td>
<td></td>
<td>1° 10.6 max 2.1 7.3 8.7 5.4</td>
<td>46 1988</td>
<td>45 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>HPLC</td>
</tr>
<tr>
<td>Rifater 2 H500mg Z300mg R120mg</td>
<td>3F 10M 58.2</td>
<td>11.5</td>
<td>1° 9.9 max 2.6 58.0 8.4 6.0°</td>
<td>15° 10.0 max 2.8 48.0 8.6 4.8°</td>
<td>39 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>HPLC</td>
</tr>
<tr>
<td>Rifater 3 6M 70.8 8.5</td>
<td></td>
<td></td>
<td>1° 6.6 max 3.0 43.0 6.4 4.8°</td>
<td>46 1993</td>
<td>47 1993</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.7 5.8 4.5 3.6 2.7</td>
<td>Sarcina lutea</td>
</tr>
</tbody>
</table>

a): Arithmetic average between 2° and 4° h. b): Arithmetic average between 4° and 8° h. c): Lower and higher figures in the set of average values from 14 studies are reported. d): Patients received also isoniazid and ethambutol intravenously. e): Arithmetic average between the two groups of 4 subjects. f): Measured on 10 subjects; AUC_{0→12} of the excluded subjects were 46.4, 43.1 and 46.6 mg h/l. g): Measured on 11 subjects; AUC_{0→12} of the excluded subjects were 44.6 and 53.0 mg h/l. h): Measured on 10 subjects; AUC_{0→12} of the excluded subjects were 47.8, 37.0 and 23.1 mg h/l. i): Measured on 5 subjects; AUC_{0→12} of the excluded subject were 26.3 mg h/l.
Table 2 - Scheme of administration and dosage (mg/kg b.w.) for the pharmacokinetics of HZR, H75mg Z300mg R150mg. The tested FDC was an experimental preparation.\textsuperscript{17}

<table>
<thead>
<tr>
<th>subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>body mass (kg)</td>
<td>80</td>
<td>78</td>
<td>67</td>
<td>100</td>
<td>63</td>
<td>64</td>
<td>94</td>
<td>73</td>
<td>82</td>
<td>63</td>
<td>69</td>
<td>62</td>
</tr>
<tr>
<td>individual drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>12.5</td>
<td>10.0</td>
<td>15.9</td>
<td>13.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>5.0</td>
<td>5.0</td>
<td>6.3</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>29.8</td>
<td>20.0</td>
<td>21.3</td>
<td>27.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>7.7</td>
<td>8.9</td>
<td>9.4</td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>free combination</td>
<td>S</td>
<td>12.5</td>
<td>12.8</td>
<td>14.9</td>
<td>10.0</td>
<td></td>
<td>16.1</td>
<td>15.8</td>
<td>14.9</td>
<td>16.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td>4.0</td>
<td></td>
<td>6.4</td>
<td>6.3</td>
<td>5.8</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>25.0</td>
<td>25.6</td>
<td>29.8</td>
<td>20.0</td>
<td></td>
<td>32.2</td>
<td>31.7</td>
<td>29.0</td>
<td>32.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>7.5</td>
<td>7.7</td>
<td>6.9</td>
<td>6.0</td>
<td></td>
<td>9.7</td>
<td>9.5</td>
<td>6.7</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fixed combination</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.8</td>
<td>15.6</td>
<td>10.6</td>
<td>13.7</td>
<td>18.2</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
<td>4.7</td>
<td>4.0</td>
<td>5.1</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.0</td>
<td>18.7</td>
<td>15.9</td>
<td>20.5</td>
<td>19.3</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.5</td>
<td>9.7</td>
<td>8.0</td>
<td>10.2</td>
<td>9.7</td>
<td>9.6</td>
</tr>
</tbody>
</table>
Table 3 - Solubility of rifampicin in water in mg/mL at different pH, data reported in the literature.

<table>
<thead>
<tr>
<th>pH</th>
<th>1</th>
<th>2.12</th>
<th>2.5</th>
<th>3.03</th>
<th>3.99</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>sol</td>
<td>200.0</td>
<td>107</td>
<td>3.60</td>
<td>1.0</td>
<td>1.0</td>
<td>0.68</td>
</tr>
<tr>
<td>ref</td>
<td>26</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>5.33</th>
<th>6</th>
<th>7</th>
<th>7.4</th>
<th>7.4</th>
<th>8.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>sol</td>
<td>0.77</td>
<td>1.4</td>
<td>1.06</td>
<td>1.53</td>
<td>9.9</td>
<td>2.12</td>
</tr>
<tr>
<td>ref</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>26</td>
<td>18</td>
</tr>
</tbody>
</table>
Figure 1 - “Plasma levels of Rimactane following oral administration of single doses of 450 mg to the same 8 subjects on three consecutive days”, first day: administration 1 hour before breakfast (closed circle, solid line), second day: administration 20 min. before breakfast (open circles, dashed line), third day: administration with breakfast (closed squares, solid lines) (left). “Plasma levels of Rimactane following oral administration of the drug to the same 6 subjects on two different days. On one day 450 mg. was given 1 hour before breakfast, and on another day the same dose was given 15 minutes after breakfast.” (right). Reproduction with kind permission of Novartis International from the original."
Figure 2 - the pharmacokinetics of HZR, H75mg Z300mg R150mg: "Plasma concentrations of rifampicin (mcg/ml) observed at the indicated time intervals (hours) after administration of R alone (closed circles, solid line), in free (asterisks, dashed line), and fixed combination with H and Z (closed squares, dashed and dotted line)." Reproduction without permission of the American Thoracic Society from the original.\textsuperscript{17}
Figure 3 – a) hydrolysis of azomethinic bond of rifampicin – b) proposed reaction between isoniazid and 1-amino-4-methylpiperazine, catalyzed by pyrazinamide – c) mechanism of the reaction in b.
Figure 4 – “Plot of the obtained first order rate constant for the acid catalyzed solvolysis of rifampicin as a function of rifampicin concentration in 0.1N HCl and 50°C” Reproduction with kind permission of S. Karger AG, Basle, from the original.²⁶
Figure 5 – Dependence of rifampicin serum concentration $\text{AUC}_{0-\infty}$ on the dose, based on data by Furesz et al.\textsuperscript{11} Reproduction with kind permission of Novartis International from the original.\textsuperscript{10}