MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 29, No. 7, pp. 733–738, 2003

RESEARCH PAPER

Behavior of Decomposition of Rifampicin in the Presence of Isoniazid in the pH Range 1–3

R. Sankar, Nishi Sharda, and Saranjit Singh*

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical
Education and Research (NIPER), S.A.S. Nagar, India

ABSTRACT

The extent of decomposition of rifampicin in the presence of isoniazid was determined in the pH range 1–3 at 37°C in 50 min, the mean stomach residence time. With increase in pH, the degradation initially increased from pH 1 to 2 and then decreased, resulting in a bell-shaped pH-decomposition profile. This showed that rifampicin degraded in the presence of isoniazid to a higher extent at pH 2, the maximum pH in the fasting condition, under which antituberculosis fixed-dose combination (FDC) products are administered. At this pH and in 50 min, rifampicin decomposed by ~34%, while the fall of isoniazid was 10%. The extent of decomposition for the two drugs was also determined in marketed formulations, and the values ranged between 13–35% and 4–11%, respectively. The extents of decomposition at stomach residence times of 15 min and 3 h were 11.94% and 62.57%, respectively, for rifampicin and 4.78% and 11.12%, respectively, for isoniazid. The results show that quite an extensive loss of rifampicin and isoniazid can occur as a result of interaction between them in fasting pH conditions. This emphasizes that antituberculosis FDC formulations, which contain both drugs, should be designed in a manner that the interaction of the two drugs is prevented when the formulations are administered on an empty stomach.

Key Words: Rifampicin; Isoniazid; FDC products; Decomposition; Fasted state.

INTRODUCTION

Studies in literature indicate that a fall in dose of rifampicin to below 9 mg/kg leads to failure of therapy and can contribute towards development of drug resistance.[1] Currently, the prescribed dose of rifampicin is 10 mg/kg (600 mg rifampicin for an average patient of 60 kg), which means a narrow margin

---

*Correspondence: Saranjit Singh, Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, 160 062, India; Fax: ++91 172 214692; E-mail: ssingh@niper.ac.in.

DOI: 10.1081/DDC-120021772
0363-9045 (Print); 1520-5762 (Online)
www.dekker.com

of only 10% in actual delivered dose and the minimum necessary for therapeutic action. Unfortunately, the drug has been reported to undergo rapid decomposition in the presence of isoniazid under acid conditions,[2,3] which means there exists a strong possibility of the dose of rifampicin falling below the minimum required level, after administration of formulations containing the two drugs in combination. Thus, apart from the initial drug content in formulation, stability of rifampicin in its dosage forms and under stomach acid conditions turns out to be an important factor in assuring therapeutic action of the drug.

Antitubercular fixed-dose combination (FDC) products are administered to patients in a fasted state where the pH ranges from 1.4 to 2.1.[4] However, all previous studies on decomposition of rifampicin in the presence of isoniazid under acid conditions report results in simulated gastric fluid or 0.1 M HCl at pH ~ 1.[2–5] Accordingly, the purpose of the present study was to determine the extent of decomposition of rifampicin in the presence of isoniazid in pure drug combinations and marketed products under fasting stomach pH conditions. However, while planning for the study, another aspect came into the picture. It was previously established that rifampicin degrades in the presence of isoniazid under acid conditions through a reaction involving formation of isonicotinyl hydrazone, which results from an interaction of isoniazid with 3-formylrifamycin, an acid degradation product of rifampicin.[6] Based upon the work by Devani et al.,[7] where a bell-shaped pH curve is reported for the parallel formation of isonicotinyl hydrazone between isoniazid and reducing sugars, it was anticipated that decomposition of rifampicin in the presence of isoniazid would also show a similar behavior, showing a fall in the decomposition curve after reaching the maximum. Hence the study was extended, to establish a pH profile for the decomposition of rifampicin in the presence of isoniazid, in the pH range 1–3. The results are reported in this communication.

MATERIAL AND METHODS

Materials and Reagents

Rifampicin, isoniazid, and 3-formylrifamycin were gift samples from Lupin Laboratories Ltd., Aurangabad, India. Hydrazone of 3-formylrifamycin and isoniazid was prepared by the method described earlier.[6] High-performance liquid chromatography (HPLC) grade methanol was purchased from Mallinckrodt Baker, Inc., Paris, KY. Buffer materials and all other chemicals were of analytical-reagent grade. Ultra-pure water obtained from an ELGA water purification unit (Elga Ltd., Bucks, England) was used.

Instrumentation

The HPLC system consisted of a dual-piston reciprocating pump (LC-10ATVP), UV-visible dual-wavelength detector (SPD-10AVP), an autoinjector (SIL-10ADVP), and an on-line degasser (DGU-14AM). The data were acquired using CLASS-VP software (all from Shimadzu, Kyoto, Japan). Decomposition reactions were carried out in precision water bath (Julabo, Seelbach, Germany).

Analytical Method

Analysis was carried out on a reversed-phase HPLC using Supelcosil LC-18-DB (25 cm × 4.6 mm, 5 μm) column and a mobile phase consisting of 65% methanol and 35% 0.01 M phosphate buffer at pH 7.00. The flow rate was maintained at 1 mL/min and the analytical wavelength was 254 nm. The method was validated for linearity, precision, accuracy, ruggedness, and specificity. For determination of linearity, stock solutions of rifampicin and isoniazid were prepared by dissolving the drugs in methanol, at a concentration of 1 mg/mL. From these stock solutions, dilutions were made in the range of 5–600 μg/mL and 5–500 μg/mL for rifampicin and isoniazid, respectively. The solutions were injected after filtering through 0.2 μm nylon membrane, keeping the injection volume constant (20 μL). The repeatability was determined by replicate ($n = 6$) injections of solutions of the two drugs at three concentrations: 5, 100, and 500 μg/mL. For the determination of intermediate precision, 300 μg/mL of rifampicin and isoniazid were prepared six times separately, and the peak areas were recorded. A study was also carried out using different instruments and chromatographic columns on different days.

Influence of pH on the Degradation of Rifampicin in the Presence of Isoniazid

A stock solution of 1 M HCl was prepared and standardized against 1 M sodium hydroxide solution, which had been standardized against 1 M oxalic acid. This stock solution was suitably diluted with water to get solutions of 0.1, 0.03, 0.01, 0.003, and 0.001 M HCl. Accurately weighed quantities of 18 mg of rifampicin and 12 mg of isoniazid (this 3/2 ratio of the two drugs was taken to maintain equivalence with the drug ratio in the marketed formulations) were added to 10 mL of each acid solution, one at a time. The solution was mixed well and a sample was withdrawn immediately. The same was suitably diluted and analyzed by HPLC. The remaining solution was maintained in a water bath at 37°C for 50 min (mean residence time in stomach). The sample was withdrawn and analyzed as above. The percentage degradation of rifampicin and isoniazid was calculated from the differences between the peak areas of samples drawn at 0 and 50 min. All the studies were done in triplicate.

The pH of the acid solutions was calculated theoretically at the study temperature, using activity coefficient data.[8]

Decomposition of Marketed Samples in Fasted-State pH Conditions

The marketed samples were available both as tablets and capsules. The tablets were weighed and crushed to a fine powder using a mortar and pestle. Capsules were emptied and the contents were subjected to study. In both cases, the amount of powder containing 18 mg of rifampicin and 12 mg of isoniazid was weighed and mixed with 10 mL of 0.01 M HCl solution. The mixtures were subjected to degradation and analysis, as given above. For comparison, decomposition was also carried out in a self-prepared physical mixture of rifampicin and isoniazid, made to the same strength. The study was carried out in triplicate in each case.

Degradation in the Fasted-State pH of 2.04 at 15 min and 3 h

As the gastric residence time of formulations may vary from 15 min to as long as 3 h, the extent of decomposition was also determined at these time periods. The studies were done in 0.01 M HCl at a pH of 2.04, very near to the maximum pH in the fasted state. Accurately weighed quantities of 18 mg of rifampicin and 12 mg of isoniazid were added to 10 mL of the acid solution. After thorough mixing, a sample was withdrawn immediately. The same was suitably diluted and analyzed. The remaining solution was maintained in a water bath at 37°C and the sample was withdrawn first at 15 min and later at 3 h and subjected to analysis. The percent degradation was calculated in the same manner as in other studies. The study was done in triplicate.

RESULTS AND DISCUSSION

The HPLC method used was able to separate standards of rifampicin, isoniazid, and degradation products, 3-formylrifamycin, rifampicin quinone, and the isonicotinyl hydrazone (Fig. 1). The resolution factor of rifampicin peak from the immediately resolving peak was greater than 2. The factor was even higher for all other resolving peaks, indicating that the method was highly selective for all the components. The response of the method was linear in the ranges of 5–600 μg/mL and 5–500 μg/mL for rifampicin and isoniazid, respectively. In total, three

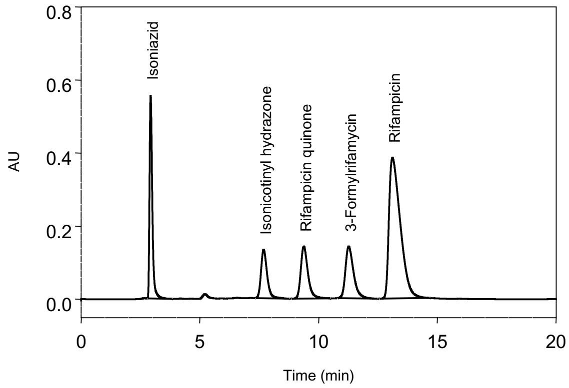

Figure 1. Chromatogram showing separation of rifampicin, isoniazid, isonicotinyl hydrazone, rifampicin quinone, and 3-formylrifamycin.

Table 1. The linearity of the HPLC method.

Drug	Linearity range ($\mu g\, mL^{-1}$)	Equation of line[a]	Correlation coefficient
Rifampicin	5–600	$y = 41220x - 22080$	0.9998
Isoniazid	5–500	$y = 34427x + 6426$	0.9999

[a]Mean of three determinations on different days.

Table 2. Recovery data for the HPLC method.

Drug	Actual concentration (μg mL^{-1})	Calculated concentration (μg mL^{-1})	Recovery (%)
Rifampicin	5	5.00 ± 0.02	100.0
	100	100.94 ± 0.28	100.9
	500	496.37 ± 1.67	99.3
Isoniazid	5	4.95 ± 0.03	99.0
	100	101.11 ± 0.25	101.1
	500	501.33 ± 0.44	100.3

Data are mean ± s.d., $n = 6$.

Table 3. Intermediate precision for the analytical method.

Drug	Actual concentration (μg mL^{-1})	Calculated concentration (μg mL^{-1})	Recovery (%)
Rifampicin	300	302.26 ± 3.39	100.8
Isoniazid	300	301.75 ± 0.48	100.6

Data are mean ± s.d., $n = 6$.

standard curves were prepared on consecutive days. The mean data are given in Table 1. The results of the precision experiments are given in Tables 2 and 3. The percent relative standard deviation (RSD) for all six injections in the repeatability and intermediate precision experiments was found to be well below the limit of 1% and 2%, respectively. The resolutions obtained during experiments using different columns and systems and on different days were satisfactory for all the compounds.

The HPLC chromatograms for the initial sample containing rifampicin and isoniazid and that degraded for 50 min at pH 2.04 are shown in Fig. 2. Evidently, isonictonyl hydrazone is formed as the major degradation product of acid decomposition of rifampicin in the presence of isoniazid.

pH-Decomposition Profile

The extents of decomposition of rifampicin and isoniazid at various pH values are given in Table 4. The corresponding pH-degradation profiles are shown in Fig. 3. Evidently, the profiles are bell-shaped with maximum decomposition occurring at pH 2.04. This is in accordance with the initial

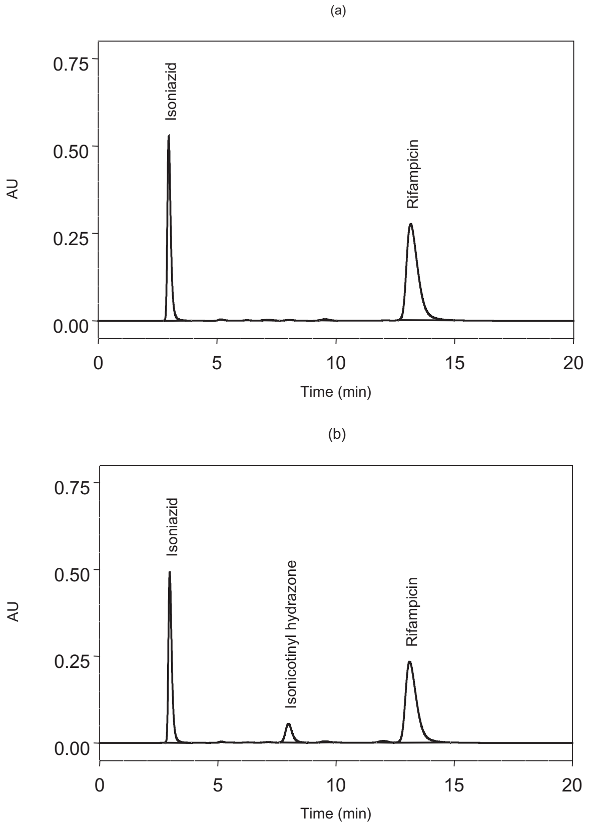

Figure 2. Chromatograms obtained for a solution of rifampicin and isoniazid in hydrochloric acid at pH 2.04. The chromatogram (a) was recorded immediately, while chromatogram (b) was obtained after keeping the solution for 50 min at 37°C. In comparison to chromatogram (a), chromatogram (b) clearly shows fall in both rifampicin and isoniazid, resulting in formation of isonicotinyl hydrazone as the major product.

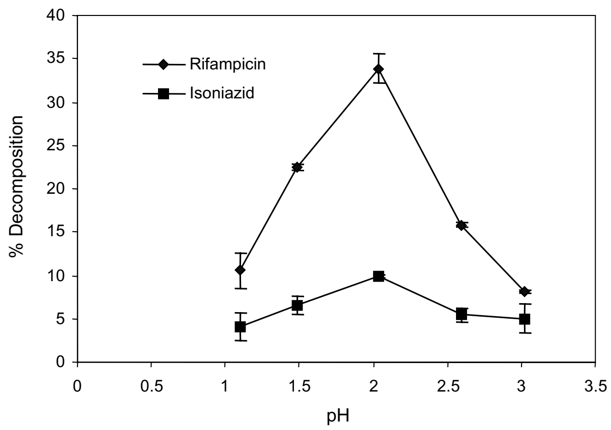

Figure 3. The influence of pH on decomposition of rifampicin and isoniazid. Each reaction solution contained both the drugs together and was kept at 37°C for 50 min.

expectation that formation of hydrazone from decomposition of rifampicin in the presence of isoniazid should run parallel to the formation of hydrazone between isoniazid and reducing sugars, where also bell-shaped behavior was observed.[7] As seen in Fig. 3, the slope of the curve is steep in the pH range 1.5–2 and then falls as the pH is increased further. This clearly indicates that the decomposition of rifampicin in the presence of isoniazid ought to be higher in the fasted state than in the fed condition. While the pH of the stomach under fasting conditions lies between 1.4 and 2.1, it is normally 3.0–5.5 in the fed state.[4] As shown in Table 4, rifampicin degrades almost three times higher at pH 2.04 than at pH 1 or pH 3.0.

Table 4. Influence of pH on decomposition of rifampicin and isoniazid, when present in combination, at 37°C for 50 min.

	Decomposition (%)	
pH	Rifampicin	Isoniazid
1.10	10.59 ± 2.03	4.01 ± 1.57
1.49	22.47 ± 0.41	6.50 ± 1.06
2.04	33.88 ± 1.62	9.98 ± 0.16
2.60	15.82 ± 0.24	5.40 ± 0.85
3.02	8.11 ± 0.20	5.04 ± 1.64

Data are mean ± s.d., $n = 3$.

Degradation of Rifampicin and Isoniazid in Marketed Two-Drug Combinations at pH 2.04

Because of the increased decomposition of rifampicin and isoniazid at pH 2.04, it was thought worthwhile to investigate decomposition of marketed formulations containing the two drugs at this pH. All marketed products containing rifampicin and isoniazid had the drug strengths of 450 mg and 300 mg, respectively, in a ratio of 3/2. The extent of decomposition of the two drugs from various formulations is given in Table 5. The percentage degradation of rifampicin apparently is between 13–35%, with a few formulations showing similar extent of decomposition to that shown by the self-prepared mixture (~34%, Table 4). The decomposition of isoniazid correspondingly ranges from 4.20 to 10.86%. The 11% loss of this drug from formulations is also similar to that shown by the mixture of pure drugs (~10%, Table 4).

The Extent of Degradation at 15 min and 3 h in Fasted-State pH Conditions

The values of percent degradation of rifampicin and isoniazid at pH 2.04 in 15 min and 3 h are given in Table 6. The given values show that depending

Table 5. Degradation studies on marketed formulations after storage in 0.01 M HCl (pH 2.04) at 37°C for 50 min.

				Degradation (%)	
Formulation code[a]	Manufacture date	Expiry date[b]	pH[c]	Rifampicin	Isoniazid
CT-1	June 1999	November 2001	2.58	13.15 ± 2.58	6.47 ± 0.63
CT-2	June 1999	May 2001	2.60	19.11 ± 0.56	7.70 ± 0.88
CT-3	May 2000	April 2002	2.57	20.82 ± 2.73	5.15 ± 0.74
CT-4	January 2000	December 2001	2.64	21.70 ± 1.63	4.20 ± 1.09
CT-5	July 2000	June 2002	2.58	21.86 ± 2.75	5.66 ± 1.61
CT-6	May 2000	April 2002	2.51	23.02 ± 5.08	7.86 ± 0.79
CT-7	December 1999	May 2001	2.59	24.42 ± 0.99	8.35 ± 1.70
CT-8	April 2000	March 2002	2.57	24.78 ± 2.06	6.76 ± 1.28
CT-9	April 2000	January 2003	2.56	24.95 ± 2.05	6.70 ± 0.70
CT-10	May 2000	April 2002	2.57	25.24 ± 3.70	5.12 ± 0.57
CT-11	August 1999	July 2001	2.55	27.18 ± 1.26	6.29 ± 1.05
CT-12	May 2000	April 2002	2.53	30.92 ± 2.88	4.91 ± 1.42
CAP	August 2000	July 2002	2.58	35.44 ± 1.78	10.86 ± 1.26

[a] CT = coated tablets; CAP = capsule.

[b] Studies were carried out between October and December 2000.

[c] Recorded immediately after addition of the drugs. These values decreased up to 0.26 pH units when solutions were stored for 50 min 37°C. pH before addition was 2.04. Data are mean ± s.d., $n = 3$.

Table 6. Loss of rifampicin and isoniazid after storage in combination in 0.01 M HCl (pH 2.04) at 37°C for 15 min and 3 h.

	Decomposition (%)	
Drug	15 min	3 h
Rifampicin	11.94 ± 2.67	62.57 ± 2.12
Isoniazid	4.78 ± 1.78	11.12 ± 1.06

Data are mean ± s.d., $n = 3$.

upon the transit time in the stomach, degradation of rifampicin in the fasted state might range from about 12% to as high as 63%. The corresponding range for isoniazid is 5–11%.

CONCLUSIONS

The study shows that rifampicin may be decomposed on administration of FDCs in fasting conditions to an extent well beyond the 10% tolerance between the delivered dose of rifampicin and the minimum necessary for therapeutic action. This loss of rifampicin, along with near equimolar amounts of isoniazid, strongly emphasizes that antitubercular FDC formulations containing rifampicin and isoniazid should be designed in a manner to provide protection against interaction between the two drugs under in situ fasting conditions. Unfortunately, there are no FDC products of the kind yet on the market. Hence the manufacturers of FDC formulations should consider revising their formulations in which the delivery of the two drugs is separated from each other, so that risk of failure of therapy is overcome.

REFERENCES

1. Long, M.W.; Snider, D.R.; Farer, L.S. US Public Health Service Cooperative Trial of Three RIF-INH Regimens in the treatment of pulmonary tuberculosis. Am. Rev. Respir. Dis. **1979**, *119*, 879–894.
2. Shishoo, C.J.; Shah, S.A.; Rathod, I.S.; Savale, S.S.; Kotecha, J.S.; Shah, P.B. Stability of rifampicin in dissolution medium in presence of isoniazid. Int. J. Pharm. **1999**, *190*, 109–123.
3. Singh, S.; Mariappan, T.T.; Sharda, N.; Singh, B. Degradation of rifampicin, isoniazid, and pyrazinamide from prepared mixtures and marketed single and combination products under acid conditions. Pharm. Pharmacol. Commun. **2000**, *6*, 491–494.
4. Dressman, J.B.; Amidon, G.L.; Reppas, C.; Shah, V.P. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. **1998**, *15*, 11–22.
5. Jindal, K.C.; Chaudhary, R.S.; Singla, A.K.; Gangwal, S.S.; Khanna, S. Dissolution test method for rifampicin-isoniazid fixed dose formulations. J. Pharm. Biomed. Anal. **1994**, *12*, 493–497.
6. Singh, S.; Mariappan, T.T.; Sharda, N.; Kumar, S.; Chakraborti, A.K. The reason for an increase in decomposition of rifampicin in the presence of isoniazid under acid conditions. Pharm. Pharmacol. Commun. **2000**, *6*, 405–410.
7. Devani, M.B.; Shishoo, C.J.; Doshi, K.J.; Patel, H.B. Kinetic studies of the interaction between isoniazid and reducing sugars. J. Pharm. Sci. **1985**, *74*, 427–432.
8. Greeley, R.S.; Smith, W.T.J.; Stoughton, R.W.; Lietzke, M.H. Electromotive force series studies in aqueous solutions at elevated temperatures. II. Thermodynamic properties of hydrochloric acid. J. Phys. Chem. **1960**, *64*, 1445–1448.