# Effect of Ascorbic Acid on dissolution stability of Rifampicin in market fixed dose combination products for Tuberculosis

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**ABSTRACT**

Degradation of rifampicin (RIF) to insoluble and poorly absorbed 3-Formyl rifamycin SV (3-FRSV) in acidic environment is a major concern which leads to reduction in the bioavailability of RIF and is further influenced by the presence of isoniazid (INH) in the stomach after ingestion. It is recommended that addition of ascorbic acid (ASC) in dissolution medium, in plasma as antioxidant to stabilize RIF from degradation and also daily intake of ASC to control tuberculosis (TB).Though the effect of ASC on fixed dose combination (FDC) products has not been traversed and hence examined in the present study. The rate of degradation of RIF to 3-FRSV in the presence of ASC in dissolution medium (0.1 N HCl) on market formulations was estimated by Dual Wavelength UV–Vis. spectrophotometry (DW spectrophotometry) and High performance liquid chromatography (HPLC) method. Addition of ASC in FDC formulations lowered significantly formation of 3-FRSV or degradation of RIF as compared to that without ASC in *in-vitro*. Our study proposed that co-package of ASC with fixed dose combination products (FDC) can protect RIF degradation in the acidic environment and *in-vivo* investigation needed to predict the bioavailability of RIF in FDC products in the presence and absence of ASC for effective control of TB.

*Keywords*: Rifampicin, ASC, 3-Formyl rifamycin SV, FDC, HPLC

## INTRODUCTION

The revival of delineated survey in tuberculosis along with the recent disclosure of multidrug resistant strains of *M*. *tuberculosis* (TB) has provoked World Health organization (W.H.O) to declare the infection as ''Global health emergency, a public health disaster''.[1] (Anon,1997). A control programme reaches the WHO targets of 70% case detection and 85% cure would reduce the incidence rate by 11% (range 8-12) per year and the death rate by 12% (9-13) per year. Without greater effort to control TB, the annual incidence of the disease is expected to increase by 41% (21-61) between 1998 and 2020 (from 7.4 million to 10.6 million cases per year). Achievement of WHO targets by 2010 would prevent 23% (15-30) or 48 million cases by 2020.[2]

Rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ) and ethambutol (ETH) are drugs of choice for the treatment of tuberculosis. They are administered either separately or as a combination dosage form. Fixed Dose Combination (FDC) of two, three or four drugs is preferred dosage regimen in India and elsewhere for better patient compliance, efficient reduction in viable bacterial population and minimizing development of resistance to anti-TB drugs. However, poor bioavailability of RIF from a number of dosage forms of RIF and its combination with INH has been reported.[3,4,5,6,7,8].

Bioavailability of RIF is known to be affected by a number of factors such as the manufacturing process, presence of food in the gastointestinal tract, acidity of the gastric juice and excipients including those used with companion drug such as p-amino salicylic acid. Ethambutol has been shown to have little or no effect on the gastrointestinal absorption of RIF and concomitant administration of INH resulted in a lower bioavailability of RIF. [9]

Degradation of RIF is pH dependent.[10] In acidic medium RIF hydrolyzes to 3-Formyl rifamycin SV (3-FRSV) and it undergoes air oxidation in alkaline medium to form inactive quinone derivative, Rifampin quinone. 3-FRSV precipitates in acidic conditions [11]. It shows high antimicrobial activity in

vitro [12] but is inactive in vivo [13]. Therefore, formation of 3-FRSV in the acidic environment of stomach can be an important factor affecting bioavailability of RIF.

Earlier study shows that ascorbic acid (ASC) administration prevents RIF degradation in plasma at ambient temperature and prolonged the stability for up to 12 hrs. Another study reports that RIF oxidizes in solution to form RIF quinone and the addition of ASC slow down this oxidation. [14]

It has been documented that antioxidants can prevent degradation of RIF in the acidic environment in the in-vitro study and the protective effect of ASC against possible adverse effects of RIF on DNA has also been reported. Therefore clinician always recommended incorporating antioxidants in the regimen of patients with tuberculosis [15]

Among the antioxidants ASC has been shown to improve the stability of RIF in acidic environment in the in-vitro study. However to our knowledge the effect of ASC on the stability of RIF in fixed dose combination has not been attempted. Thus the present study reports our results on exploration of RIF co-administered with ASC as stabilizing agent can improve the stability of RIF in fixed dose combination by *in-vitro* studies. The outcome of the study may help to design a novel pharmaceutical approach to control effectively TB.

## MATERIALS AND METHODS

**Materials:** Rifampicin, 3-FRSV and isoniazid were procured from Themis lab, Mumbai. Pyrazinamide, ethambutol was purchased from Lupin Pharma, Mumbai. Ascorbic acid was procured from Celin, GlaxoSmithKline, Bangaluru, Karnataka. Chloroform was purchased from SRL chem, Mumbai. Concentrated hydrochloric acid was procured from Ranbaxy Ltd, Vijayawada. Potassium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate were purchased from S.D fine chemicals, Bangalore. Sodium hydroxide was procured from Loba chemicals, Mumbai. Acetonitrile (ACN) was purchased from Samir tech chem, Mapukur. Marketed formulations were procured from Novarties, Sandoz and Lupin, Chikaldana, Aurangabad, India.

### METHODS

**Dissolution stability study**

Dissolution stability study was performed on RIF alone, RIF-INH, RIF-INH-ASC combination in pH 1.2 medium. A solution of 0.1 N HCl (200 ml) was placed in the vessel of the USP dissolution apparatus 2 (USP XXIII, 1995) and the medium was equilibrated at 37 ± 0.2°C with stirring at 100 rpm. 150 mg RIF, 100 mg INH and 500mg ASC were selected for the study. Sample was accurately weighed, dissolved in and diluted to 100 ml with 0.1 N HCl (37°C). The resulting solution was transferred immediately to the dissolution vessel at once and 5 ml of specimen was withdrawn immediately from a zone midway between the surface of the dissolution medium and bottom of the vessel (0 min sample). The aliquot withdrawn for analysis was replaced with equal volume of fresh dissolution medium at 37 ± 0.2°C. Samples were withdrawn at 15 min intervals up to 60 min. The experiment was performed in triplicate.

**Estimation of RIF**

An aliquot, 1 ml was extracted immediately with 5 ml of chloroform using cyclomixer (3 min). The aqueous phase was discarded and anhydrous sodium sulphate was added to chloroform layer to remove traces of water. The sample was analyzed for RIF and 3-FRSV by DW-Spectrophotometric method at their characteristic wavelength (475–507)[16] (Shimadzu 160A UV-Vis, Japan). The percent dissolution of RIF and percent formation of 3-FRSV were determined. From the percent dissolution of RIF percent degradation of RIF was calculated from the following equation: % degradation of RIF = (Initial concentration−Final concentration) / Initial concentration× 100.

**Estimation of INH**

An aliquot, 1 ml was made up to 10 ml with dissolution medium and drug content was determined by using UV–Vis spectrophotometer at 263 nm [17] (Shimadzu UV-1800, Japan).

**Dissolution study of marketed FDC formulations (RIF, INH, ETH, PYZ)**

Dissolution medium (0.1 N HCl; 900 ml) was placed in the vessel of the apparatus [USP apparatus No. 1 (Basket)] for tablet (USP XXIII, 1995). The apparatus was assembled and the dissolution medium was allowed to equilibrate to 37±0.2°C. The FDC tablet was placed in the vessel (basket) taking care to exclude air bubble from the surface of the dosage form unit and the apparatus was operated immediately at 100 rpm. This experiment was performed in the presence and absence of ASC tablet (500mg). Aliquot sample, 5 ml, was withdrawn at an interval of 15 mins from the zone midway between the surface of the dissolution medium and top of the rotating blade of the basket (0 min sample) up to 45 min. The aliquot withdrawn for analysis was replaced with equal volume of fresh dissolution medium at 37 ± 0.2°C. The experiment was performed in triplicate.

An aliquot, 1 ml, was extracted immediately with 5 ml of chloroform using cyclomixer (3min). The aqueous phase was discarded and chloroform extract was dried over anhydrous sodium sulfate. The withdrawn samples were analyzed by validated high performance liquid chromatography (HPLC) [18] (Shimadzu CLASS- SPD-M10A VP, Japan) for estimation of RIF, 3-FRSV, INH, ETH and PYZ.

**Chromatographic conditions**

The parameters specified by USP for gradient HPLC analysis of RIF, INH, 3-FRSV are given in Table 1. The prescribed gradient program for analysis is given in Table 2. The analysis was done maintaining all the specified conditions. [18]

**Table 1: HPLC parameters for determination of FDC formulations by the proposed USP method.**

| Parameter | Condition |
|---|---|
| Column | 4.6mm×25cm |
| Particle size | 5µm |
| Mobile phase | Phosphate buffer (6.8 pH) (A)<br>Acetonitrile (B) |
| Flow rate | 1.5ml/min |
| Detection wavelength | 238nm |
| Column temperature | 25c |
| Injection volume | 20µl |

**Table 2: Gradient program for determination of FDC formulations by prescribed in the USP method.**

| Time (min) | Solution A (%) | Solution B (%) | Elution |
|---|---|---|---|
| 0 | 100 | 0 | Equilibration |
| 0-5 | 100 | 0 | Isocratic |
| 5-6 | 100-0 | 0-100 | Linear gradient |
| 6-15 | 0 | 100 | Isocratic |

## RESULTS AND DISCUSSION

The report of our study demonstrates that co-administration of ASC with market FDC product can reduce the degradation of RIF. In earlier studies followed a dual wavelength spectrophotometric analysis for simultaneous estimation of RIF and 3-FRSV in the dissolution medium.[19]

The *in-vitro* dissolution stability study was performed on RIF, 3-FRSV, other first line anti TB drugs such as INH, ETH, PYZ and on marketed FDC product in the presence and absence of ASC. Best of our knowledge no study has been performed on FDC market formulations and ASC effect on its. Our finding shows that time dependent increase in the degradation of RIF alone and in the presence of INH. The percent degradation of RIF was 13.39 at 60min and it was increased to 20.8 in the presence of INH (Table 3.). It was documented that INH accelerates the degradation of RIF in FDC products and also in our previous study it was observed that RIF degradation was influenced by INH from 13.35 - 21.4%.[20]

The effect ASC on RIF degradation and formation of 3-FRSV shown in Table 4. There was graded increase in the formation of 3-FRSV at different time point intervals and maximum formation of 3-FRSV was 21.24% in the presence of INH, compared to RIF alone (10.87%) at 60min. A decrease in 3-FRSV formation was found in addition of ASC which indicates reduction in RIF degradation. ASC significantly reduced degradation of RIF or formation of 3-FRSV in the absence as well as in the presence of INH. However there was no significant influence by the other two first line drugs such as ETH and PYZ on the degradation of RIF in acidic medium.

Table 3.
Effect of ASC on degradation of RIF in pH 1.2 medium by DW-Spectrophotometry
(Mean±S.D, n=3)

| | | | % degradation of RIF | | |
|---|---|---|---|---|---|
| Time (min) | RIF | RIF-INH | RIF-INH-ASC | RIF-INH-ETH-PYZ | RIF-INH-ETH-PYZ-ASC |
| 0 | - | - | - | - | - |
| 15 | 3.32 ±0.130 | 5.643±0.030 | 1.46±0.025 | 5.324±0.012 | 1.501±0.136 |
| 30 | 8.51±1.009 | 9.56±0.030 | 4.07±0.037 | 8.987±0.140 | 4.21±0.254 |
| 45 | 10.60±1.707 | 15.36±0.010 | 6.46±0.034 | 15.94±0.134 | 5.98±0.016 |
| 60 | 13.39±1.111 | 20.86±0.020 | 8.92±0.034 | 21.95±0.056 | 9.18±0.147 |

Table 4.
Effect of ASC on percent formation of 3-FRSV from RIF degradation in pH 1.2 medium by DW-Spectrophotometry
(Mean±S.D, n=3)

| | | | % formation of 3-FRSV | | |
|---|---|---|---|---|---|
| Time(min) | RIF | RIF-INH | RIF-INH-ASC | RIF-INH-ETH-PYZ | RIF-INH-ETH-PYZ-ASC |
| 0 | 0 | - | - | - | - |
| 15 | 2.22±0.020 | 7.643±0.014 | 0.98±0.01 | 7.924±0.001 | 1.06±0.004 |
| 30 | 6.41±0.051 | 11.56±0.002 | 3.57±0.041 | 11.95±0.024 | 3.28±0.002 |
| 45 | 8.66±0.030 | 15.96±0.004 | 6.35±0.002 | 16.23±0.018 | 6.95±0.035 |
| 60 | 10.87±1.70 | 21.24±0.030 | 7.92±0.025 | 20.53±0.001 | 6.81±0.051 |

Table 5.
Dissolution stability study of marketed FDC formulations by HPLC at 60 min.
(Mean±S.D, n=3)

| Market formulations | % RIF released | % 3-FRSV formed |
|---|---|---|
| Sample A[a] | 92.52 | 08.23 |
| Sample A[a]- 500mg ASC Tablet | 97.05 | 04.56 |
| Sample B[b] | 80.58 | 20.13 |
| Sample B[b]- 500mg ASC Tablet | 89.45 | 12.62 |
| Sample C[c] | 81.03 | 20.96 |
| Sample C[c]- 500mg ASC Tablet | 91.34 | 07.77 |

a - Formulation containing RIF (450mg)
b - Formulation containing RIF (450mg), INH (300mg)
c - Formulation containing RIF (450mg), INH (300mg), PYZ (750mg), ETH (800mg)

Dissolution study of marketed formulations of RIF alone and RIF in combination with INH and ETH, PYZ was carried out by HPLC method. The results of % degradation and % formation of 3-FRSV from market FDC formulations were shown in Table 5. This study was performed in the presence and absence of ASC Tablet (500mg). The RIF–INH combination formulations show significant amount degradation of RIF as compared to the formulations containing RIF alone (92.52-80.58%). The amount of 3-FRSV formed was in the range of 8.23–12.62% over the period of 60 min. Addition of ASC decreased % degradation (92.52- 97.05) and % 3-FRSV formation (8.23-4.56). Thus the report indicates increases in % 3-FRSV formation and % degradation of RIF at different time point intervals were significantly reduced by addition

of ASC. The above findings clearly indicate the stabilizing effect of ASC against RIF degradation in FDC products in the acidic medium of the stomach.

ASC is being employed as anti-oxidant in in-vitro dissolution medium contains FDC products for estimation of RIF in order to protect RIF from oxidative degradation during the process and thus it can be proposed that the anti-oxidant effect of ASC may be contributing to improved stability of RIF in the presence of INH, ETH, PYZ in the acid medium. Furthermore it was observed that there was no significant influence of ETH, PYZ on RIF degradation in FDC products.

## CONCLUSION

The results of the study reveal that ASC can significantly reduce the degradation of RIF from FDC products in acidic environment of the stomach and further study is need to observe the bioavailability of RIF from FDC. Besides co-administration of ASC with FDC products consists of RIF, INH, PYZ and ETH always appreciable by the clinician to improve patient's compliance and adherence to tuberculosis therapy.

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