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Full Length Research Paper

Stability- indicating (liquid chromatographic) LC method for the determination of rifabutin in bulk drug and in pharmaceutical dosage form

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A novel stability-indicating LC assay method was developed and validated for quantitative determination of Rifabutin in bulk drugs and in pharmaceutical dosage form in the presence of degradation products generated from forced degradation studies. An isocratic, reversed phase LC method was developed to separate the drug from the degradation products, using an Ace5-C18 (250 x 4.6 mm, 5 µm) column, and 50 mM ammonium acetate (pH- 4 by acetic acid) and acetonitrile (50:50v/v) as a mobile phase. The detection was carried out at the wavelength of 275 nm. The Rifabutin was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Degradation was observed for Rifabutin hydrolysis (acid, base), oxidation and photolysis conditions attempted. There is no degradation in thermal condition. The degradation products were well resolved from the main peak. The percentage recovery of Rifabutin ranged from (99.42 to 100.27%) in pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies prove the stability-indicating power of the method.

Key words: Rifabutin, column liquid chromatography, stability indicating method, validation.

INTRODUCTION

Rifabutin is chemically known as (Figure 1; 4-deoxo- 3, 4-[2-*spiro*-(*N*-iso- butyl - 4 - piperidyl) - 2, 5 - dihydro - 1H - imidazo] - rifamycin-S), a semisynthetic derivative of rifampicin S, that has shown broad-spectrum antibacterial activity against Gram-positive and Gram-negative organisms, including mycobacteria. Rifabutin was found to have activity against Mycobacterium avium-intracellular isolated from patients with AIDS (Heifets, 1987) has been approved for prophylaxis of combination therapy containing rifampin.Disseminated *Mycobacterium avium* complex (MAC) in patients infected with human immunodeficiency virus (HIV). Prophylactic treatment with rifabutin was shown to decrease the incidence of MAC by approximately 50% in AIDS patients enrolled in two randomized, placebo - controlled clinical trials (Nightingal, 1993). Rifabutin (Rfb) is a bactericidal antibiotic drug primarily used in the treatment of tuberculosis. Its effect is based on blocking the DNA-dependent RNA-polymerase of the bacteria. It is effective against Gram-positive and some Gram-negative bacteria, but also against the highly resistant Mycobacteria, e.g. *Mycobacterium tuberculosis*, *Mycobacterium leprae* and *Mycobacterium avium intracellulare.*

A recent literature survey revealed that few methods were available for the determination of rifabutin in biological samples, which involved high performance liquid chromatography (HPLC) with UV detection (Lau, 1996; Gatti, 1999). According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Literature survey reveals that there is no stability-indicating LC assay method for determination of Rifabutin in bulk drug and pharmaceutical dosage form. In the present research article, we report the development and validation of a stability-indicating LC method for the determination of

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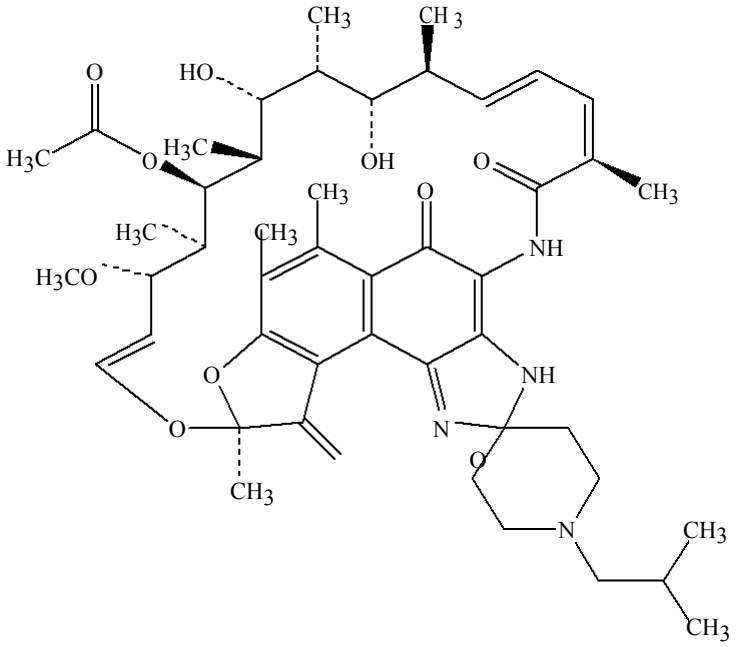

Figure 1. Chemical structure of rifabutin.

Rifabutin as bulk drug and pharmaceuti-cal dosage form. It separates drugs from the degradation products under ICH suggested stress conditions (hydrolysis, oxidations, photolysis and thermal stress) (Bakshi 2001). We developed a rapid, robust and economic method, which separates the degradation products from the main peak. The developed method is stability indicating and can be used for assessing the stability of Rifabutin in bulk drugs and pharmaceutical dosage form. The developed method was validated with respective linearity, accuracy, precision, LOD, LOQ and robustness.

EXPERIMENTAL

Material and reagents

Rifabutin bulk drug (purity 99.8) was obtained from Lupin Pharmaceutical (Aurangabad India) and ributin 20 capsules (150 mg) (manufactured by Lupin Pharmaceutical, India) were obtained from the market. Ammonium acetate and hydrochloric acid were obtained from Qualigens Fine Chemicals, India. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem Laboratories, India. All chemicals and reagents used were of analytical or LC grade. UV cabinet was used of Desaga made, (Germany). Milli-Q-Water was used throughout the experiment.

Chromatographic conditions

LC system used was a Jasco (2000 series), system equipped with a UV detector. The chromatographic column Ace5-C18 (250 x 4.6 mm, 5 µm, Advance Chromatography Technology, USA) was used. The instrumental setting was at a flow rate of 1 ml min^{-1}. The injection volume was 20 µl. The detection wavelength was 275 nm.

Mobile phase

The mobile phase consists of buffer and acetonitrile in the ratio of (50:50 v/v). The buffer used in the mobile phase contained 50 mM of ammonium acetate in double-distilled water (pH 4 by acetic acid). The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed.

Preparation of standard stock solution

All solutions were prepared on weight basis and solution con-centrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of Rifabutin was prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluent A was methanol and diluent B was mobile phase. 5 mg of rifabutin was accurately weighed, transferred to a 50 ml volumetric flask, dissolved and diluted to 50 ml with diluent A. From this stock solution 1 ml solution diluted upto 10 ml with diluent A, again from this stock solution 1 ml diluted upto 10 ml with diluent B. This final solution contained 1 g/ml of Rifabutin.

Sample solution (tablets)

Content of twenty capsules of Rifabutin (150 mg) were finely ground using agate mortar and pestle. The ground material, equivalent to 5 mg of the active pharmaceutical ingredient, was extracted into diluent A in a 50 ml volumetric flask by vortex mixing followed by ultra sonication and made up to volume by diluent B. The solution was filtered through a 0.45- micron filter and an appropriate concentration of sample (1 g/ml assay concentration) was prepared in diluents at the time of analysis.

Specificity/selectivity

Specificity is the ability of the method to assess unequivocally the

analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix, etc. (ICH Guidelines). The specificity of the developed LC method for Rifabutin was carried out in the presence of its degradation products. Stress studies were performed for Rifabutin bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (1 N hydrochloric acid), alkali (1 N NaOH), hydrogen peroxide (30%), heat (80°C) and UV light (254 nm and 366 nm wavelength) to evaluate the ability of the proposed method to separate Rifabutin from its degradation products. For light and heat studies, the study period was 24 h whereas for acid, oxidation and for base 4 h. Peak purity of the test was carried out for Rifabutin by using a UV detector in stress samples. Assay studies were carried out for stress samples against rifabutin reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degradants) was calculated. The excipient mixture present in (Ributin) 20 capsules was injected in the optimized conditions to show the specificity of the method in formulation of rifabutin.

Forced decomposition studies

Acidic degradation

About 1 mg of rifabutin was accurately weighed and dissolved in 1 ml of diluent A, then 2 ml of 1 N HCl were added and kept at room temperature for about 4 h and then the solution was neutralized by 1 N NaOH to pH 7 and the volume made up to 10 ml with diluent B. The solution was prepared to achieve a final concentration of 1 g/ml.

Alkali degradation

About 1 mg of rifabutin was accurately weighed and dissolved in 1 ml of diluent A, then 2 ml of 1 N NaOH were added and kept at room temperature about 4 h. Then the solution was neutralized by 1 N HCl to pH 7 and the volume made up to 10 ml with diluent B. The solution was prepared to achieve a final concentration of 1 g/ml.

Oxidative degradation

About 1 mg of rifabutin was accurately weighed and dissolved in 1 ml of diluent A, and then 2 ml of 30% H_2O_2 solution was added and kept at room temperature for about 4 h. Then the volume was made up to 10 ml with diluent B the solution was prepared to achieve a final concentration of 1 g/ml.

Thermal degradation

About 5 mg of drug substance was kept at 80°C for 24 h then the solution was prepared to achieve a final concentration of 1 g/ml.

UV degradations

About 5 mg of drug substance was exposed to UV short (254 nm) and UV long (366 nm) light for 24 h. Then the solution was prepared to achieve a final concentration of 1 g/ml.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability-indicating LC method was to achieve the resolution between rifabutin and its degradation products. To achieve the separation of degradation products, stationary phases of C-18 and a combination of mobile phase 50 mM ammonium acetate with acetonitrile were used. The separation of degradation products and rifabutin was achieved on an Ace5, C-18 column and 50 mM ammonium acetate pH 4 by acetic acid: acetonitrile (50:50 v/v) as a mobile phase and a column temperature at 30°C. The tailing factor obtained was less than 2 and retention time was also near about 7.5 min for the main peak and between 6 to15 min for degradation products, which would reduce the total run time and ultimately increase productivity thus reducing the cost of analysis per sample. The forced degradation study showed the method was highly specific and the entire degradation products were well resolved from the main peak. The developed method was found to be specific and validated as per ICH guidelines.

Result of forced degradation experiments

Degradation was observed at hydrolysis (acid and alkali), oxidative, thermal and UV stress conditions. Rifabutin was degraded into all attempted stress conditions (Figures 2a to f). The acceptance criterion for stability of Rifabutin is 20 to 80% degradation for forced degradation study. In acidic condition rifabutin degraded up to 11.77%, in basic condition up to 9.99%, in oxidative condition 35%, in thermal condition there is no degradation and in light condition 30.88% degradation was observed for rifabutin (Table 1). Peak purity results greater than 990 indicate that the Rifabutin peak is homogeneous in all stress conditions tested. The mass balance of Rifabutin in stress samples was close to 100% and moreover, the unaffected assay of Rifabutin in capsules confirms the stability-indicating power of the method.

Method validation

Precision

Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of test samples of rifabutin against reference standard. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different LC systems and different days in the same laboratory. The percentage of RSD and six assay values obtained by two analysts were 0.26, 99.21 and 0.31, 99.1 respectively (Table 2).

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed

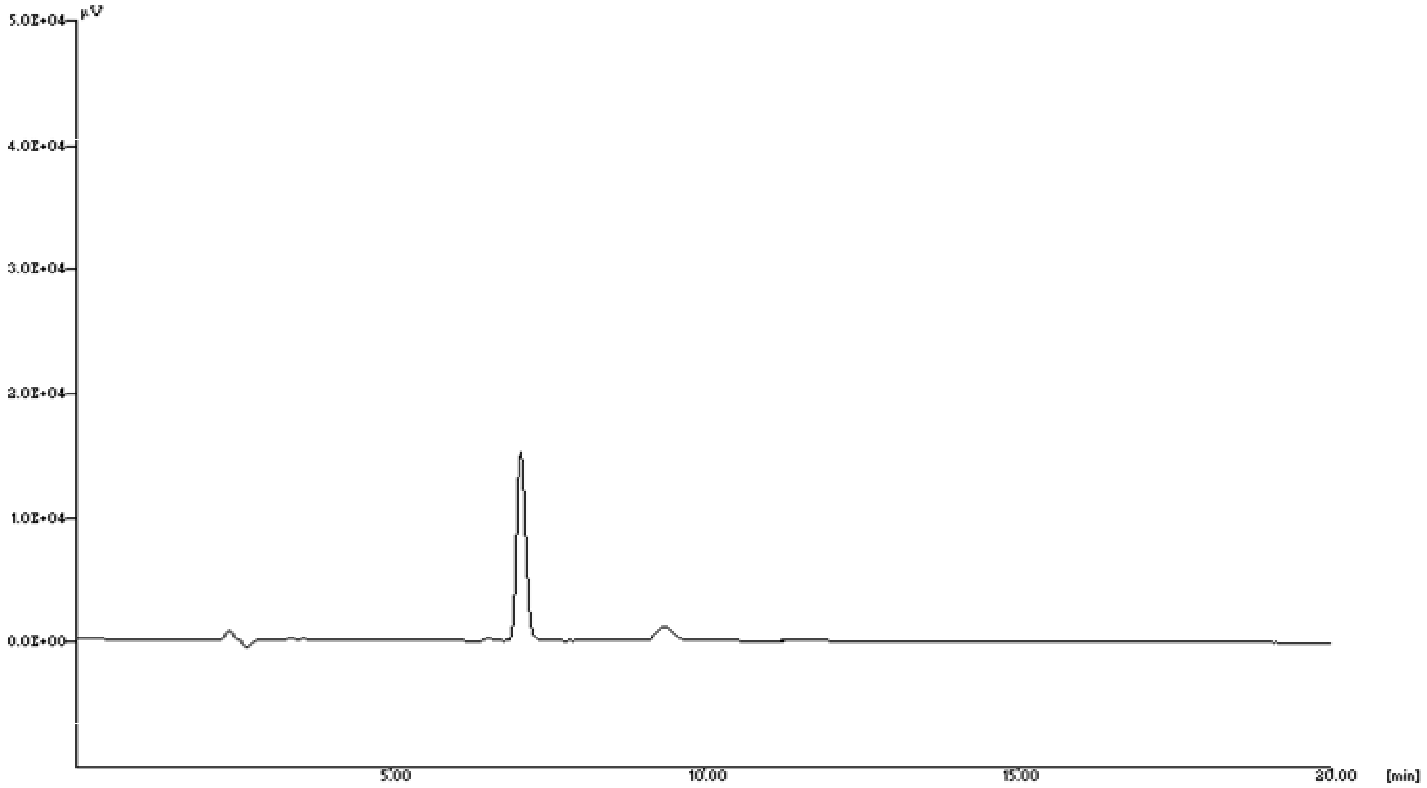

Figure 2a. Chromatogram of rifabutin in acid degradation (acid degraded product (9.783) and rifabutin (7.075).

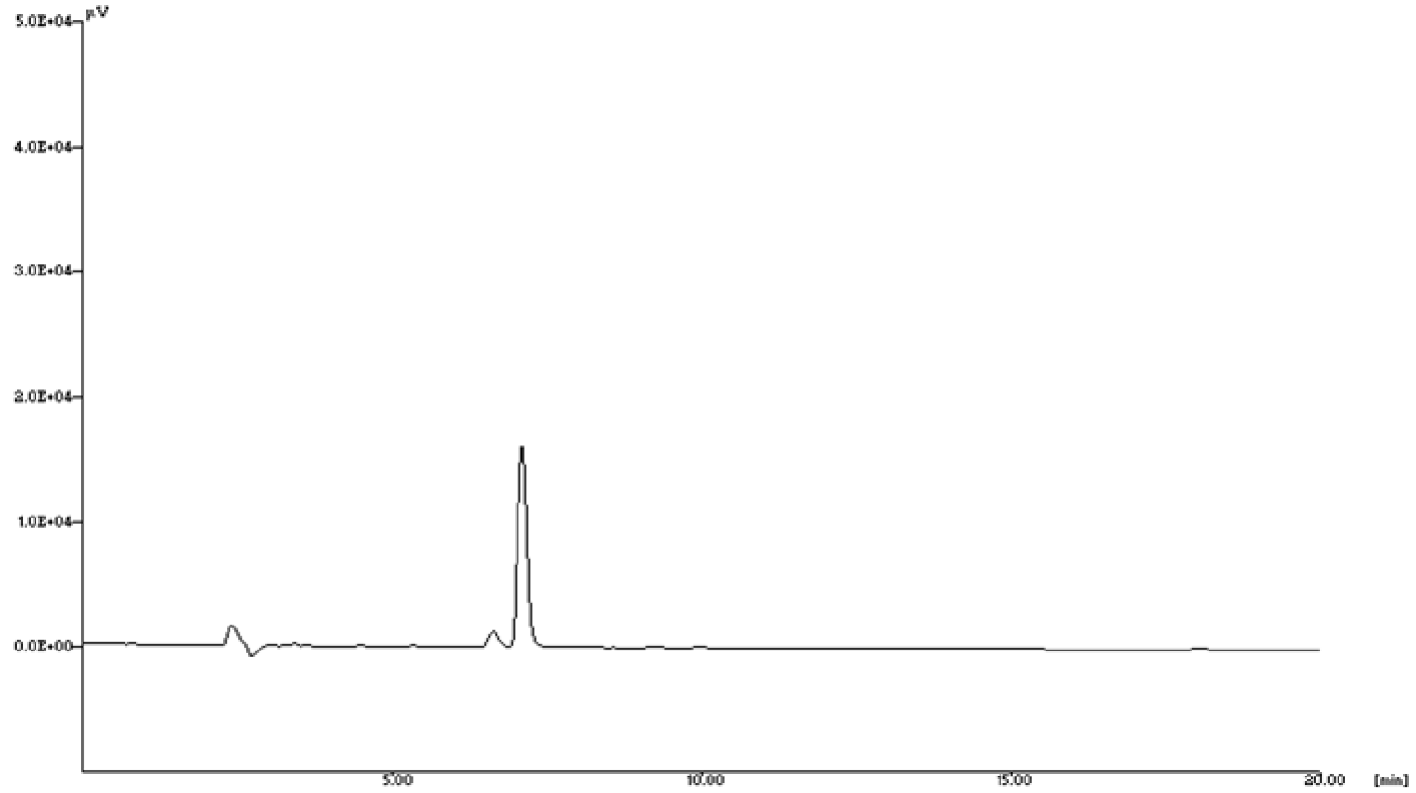

Figure 2b. Chromatogram of rifabutin in base degradation (base degraded product (6.633) and rifabutin (7.092).

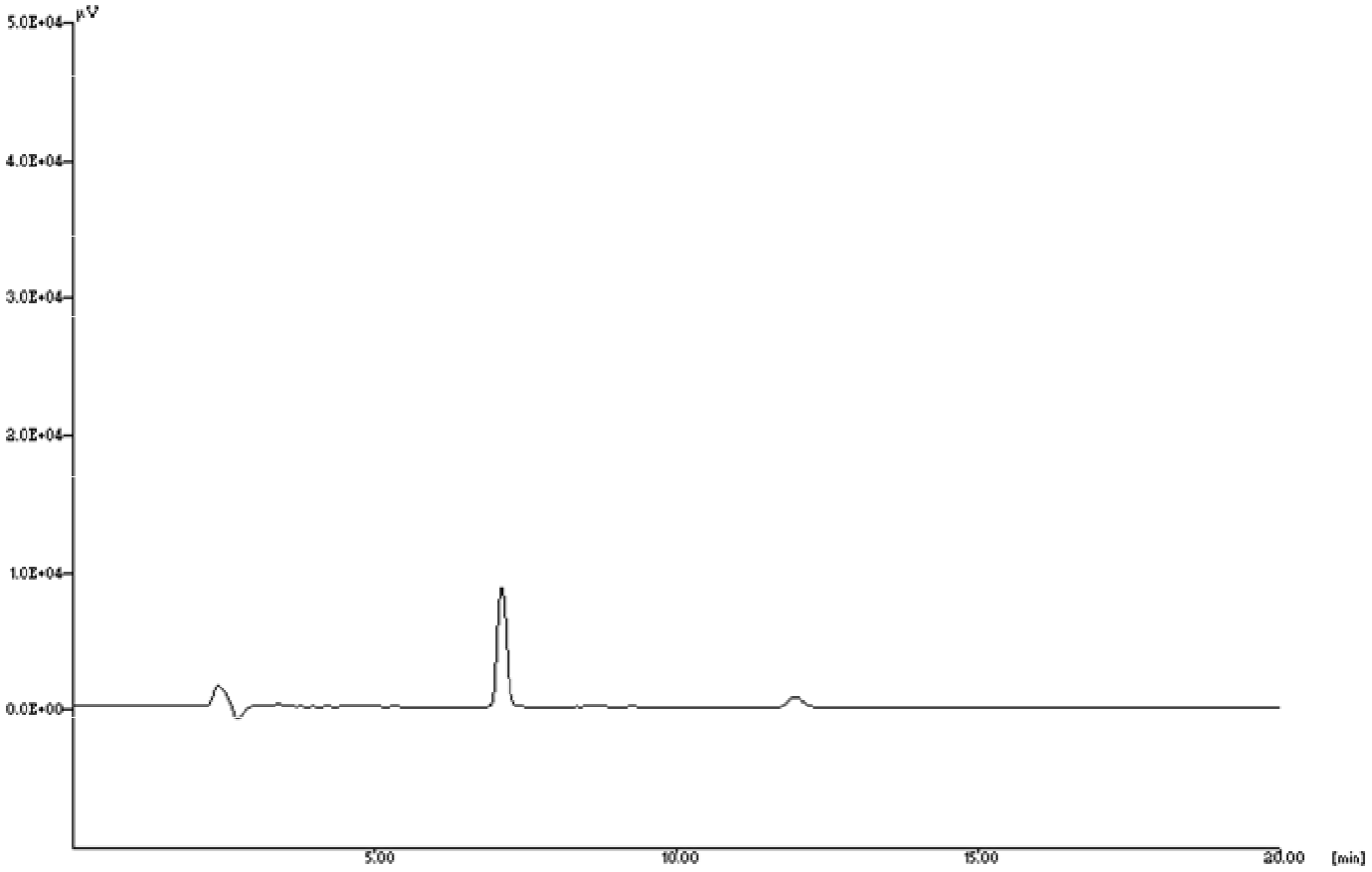

Figure 2c. Chromatogram of rifabutin in oxidative degradation (oxidative degraded product (14.267) and rifabutin (7.533).

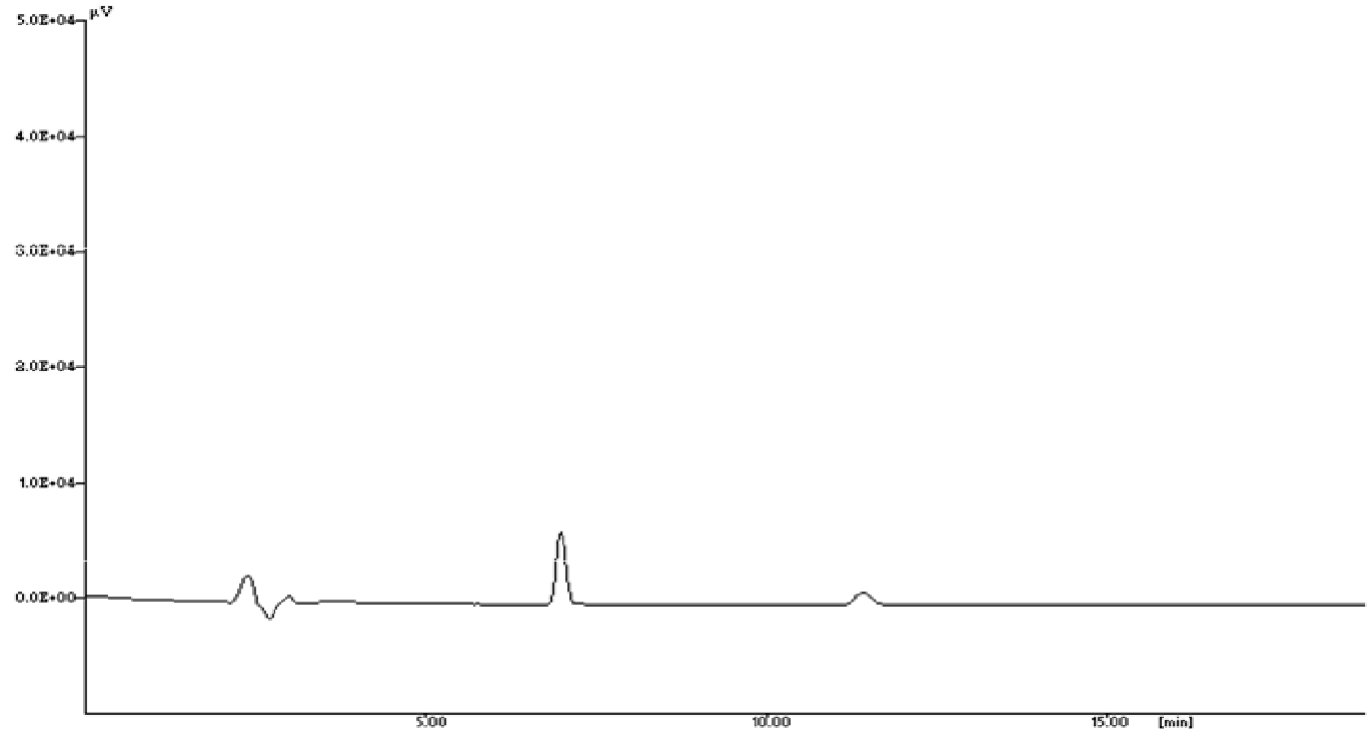

Figure 2d. Chromatogram of rifabutin in UV degradation (UV degraded product (11.392) and rifabutin (6.958).

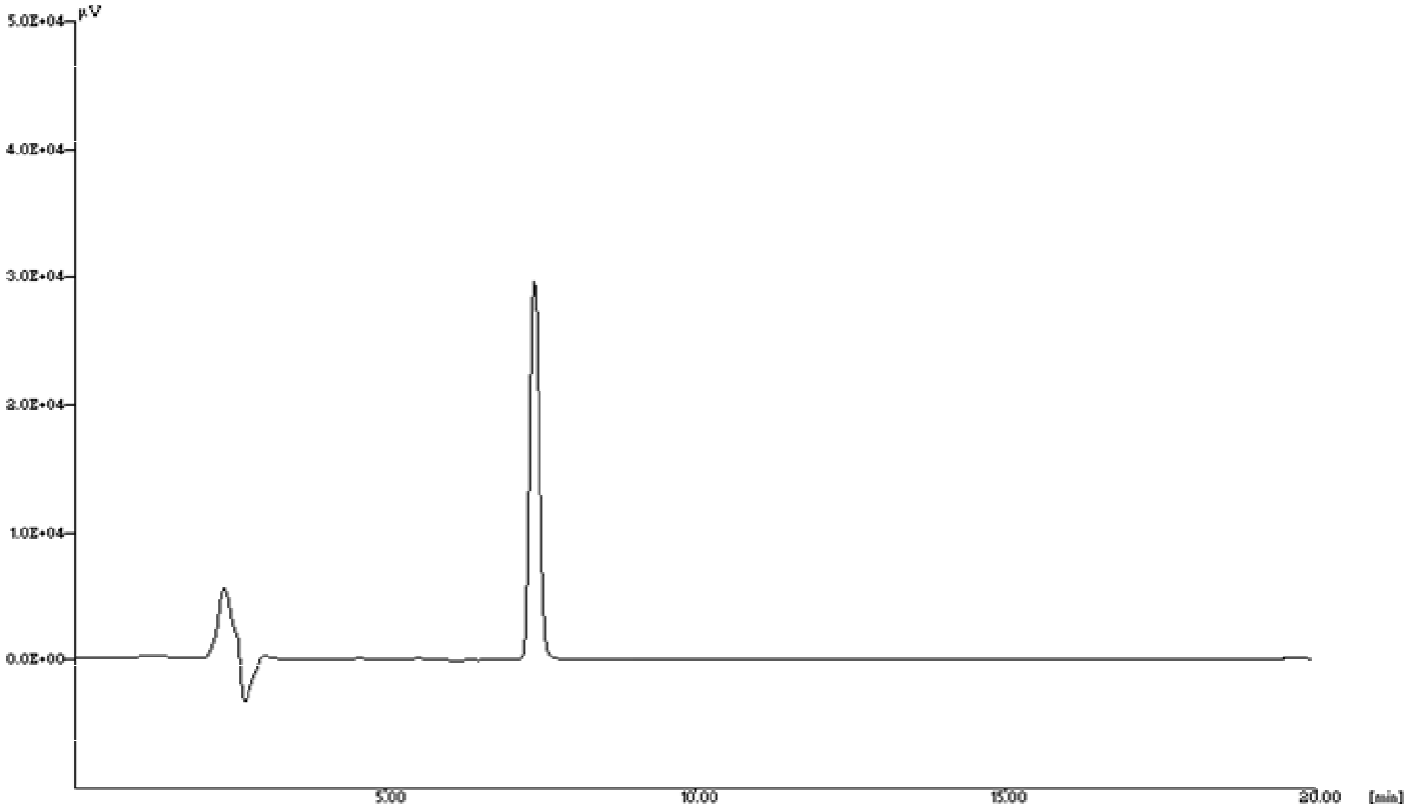

Figure 2e. A typical chromatogram of rifabutin (7.392) in the capsule.

Table 1. Summary of Forced degradation results.

Stress conditions	Time (h)	Assay of active substance %	Mass balance (% assay + % sum of impurities + sum of all degradants)	% Degradation
Acid hydrolysis (1 N HCl)	4	88.12	99.89	11.77%
Base hydrolysis (1 N NaOH)	4	89.79	99.78	9.99%
Oxidation (30% H_2O_2)	4	64.47	99.47	35%
Thermal (80°C)	24	99.79	99.79	No degradation
Light (UV 254 nm and 366 nm)	24	68.77	99.65	30.88%

By adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80,100 and 120% of the label claim of the capsules (150 mg of rifabutin). The recovery samples were prepared in the afore mentioned procedure, and then 5 ml of rifabutin solutions were transferred into a 50 ml volumetric flask and the volume made up with diluents B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for rifabutin ranged from 99.42 to 100.27%.

Linearity

The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 200% of the targeted level (1 g/ml), of the assay concentration. Standard solutions containing 0.1 to 2 g/ml of rifabutin in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting the peak area versus the concentration data and were treated by least-squares linear regression analysis. The equation of the calibration curve for rifabutin obtained y = 96027x + 4483.9, the calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination was 0.999.

Limit of detection and limit of quantification

The LOD and LOQ of rifabutin were determined by using

Table 2. Result of precision of test method.

Sample number	Assay of rifabutin as % of labeled amount	
	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)
1	99.32	99.25
2	99.45	99.87
3	99.89	99.56
4	99.49	99.10
5	99.78	99.87
6	99.21	99.43
Mean	99.21	99.1
RSD	0.26	0.31

Table 3. Results of the recovery tests for the rifabutin.

Level of addition (%)	Ingredient	Amount added (n = 3) (mg)	Recovery (%)	Average recovery (%)
80	Rifabutin	6	99.42	99.42
100	Rifabutin	8	100.27	
120	Rifabutin	10	99.92	

signal to noise approach as defined in international conference on harmonization (ICH) guidelines. Increasingly dilute solution of each drug was injected into the chromatograph and signal to noise (S/N) ratio was calculated at each concentration. The LOD and LOQ for Rifabutin in the LC method was 0.04 and 0.5 µg ml^{-1} respectively.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between rifabutin and acid degradation products were evaluated. The flow rate of the mobile phase was 1.0 ml min^{-1}. To study the effect of flow rate on resolution, it was changed by 0.2 units from 0.8 to 1.2 ml min^{-1}. The effect of percent organic strength on resolution was studied by varying acetonitrile from -10 to +10%. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C, while the other mobile phase components were held constant in chromategraphic condition. The resolution in the robustness study was not less than 5 in all conditions.

Stability of analytical solution

The stability of the standard solutions and the sample solutions were tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions.

The RSD for the assay results determined up to 72 h for Rifabutin was 0.77%. The assay values were within 1.5% after 72 h. The results indicate that the solutions solutions were stable for 72 h at ambient temperature.

DISCUSSION

From the stress study it is observed that the drug is more unstable in thermal and light condition compared with other stress conditions like acidic and basic.

Determination of active ingredients in tablets

The validated LC method was applied to the determination of rifabutin in capsules. Three batches of the capsules were assayed and the results are shown in (Table 3) indicating that the amount of drug in capsules samples met with requirements (90 to 110% of the label claim). The chromatogram of the capsules sample is shown in (Figure 2e).

CONCLUSIONS

The developed method is stability indicating and can be used for assessing the stability of rifabutin in bulk drugs and pharmaceutical dosage form. The developed method can be conveniently used for the assay determination of Rifabutin in bulk drugs and pharmaceutical dosage form. The developed LC method was specific, selective, robust,

rugged and precise. The developed LC method can be conveniently used for assessing stability, assay, related substances and dissolution of capsules of the pharmaceutical dosage form containing rifabutin in quality control laboratories as previously reported.

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