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Research Article
Stability-indicating UV-spectrophotometric Assay of Rifampicin

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Abstract

Background: Rifampicin (RIF), chemically known as 3-[[(4-methyl-1-piperazinyl)-imino]-methyl]-rifamycin SV is potentially hepatoxic and is an established first-line antituberculosis agent derived from rifamycin SV and its use in other serious infections, such as HIV is increasing. **Objective:** Two simple, sensitive, precise and economical UV-spectrophotometric methods were developed and validated for the determination of rifampicin (RIF) in bulk drug, capsule formulation and spiked human urine. **Materials and Methods:** The methods are based on the measurement of absorbance of RIF either in 0.1 M hydrochloric acid (HCl) at 263 nm (method A) or in 0.1 M orthophosphoric acid (H_3PO_4) at 259 nm (method B). **Results:** The methods were validated for linearity, accuracy and precision, limits of detection (LOD) and quantification (LOQ) and robustness and ruggedness as per the current ICH guidelines. Beer's law was obeyed over concentration range of 1.5-30 µg mL^{-1} RIF in both methods with correlation coefficients of 0.9995 and 0.9997 for method A and method B, respectively. The corresponding molar absorptivity values were 2.49×10^4 and 2.71×10^4 L mol^{-1} cm^{-1}. The utility of the methods was tested by their application to marketed formulation and the relative error and relative standard deviations were less than 2.1%. The validity and reliability of the proposed methods were further ascertained by the recovery studies via standard addition technique. In addition, forced degradation of RIF was conducted in accordance with the ICH guidelines. Acidic, basic, thermal, peroxide and photolytic stress conditions were used to assess the stability-indicating potency of the methods. **Conclusion:** Very slight degradation under peroxide-induced, slight degradation under acid-induced and substantial degradation under base-induced stress conditions were observed in both methods. No degradation was observed under other stress conditions. To enhance their usefulness, the methods were successfully applied to the determination of RIF in spiked human urine with satisfactory recovery.

Key words: Rifampicin, UV-spectrophotometry, stability-indicating, spiked human urine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rifampicin (RIF) (Fig. 1), chemically known as 3-[[(4-methyl-1-piperazinyl)-imino]-methyl]-rifamycin SV is potentially hepatoxic and is an established first-line antituberculosis agent derived from rifamycin SV and its use in other serious infections, such as HIV is increasing[1]. It is metabolized in the liver mainly by deacetylation and is excreted with its metabolites in bile. This requires careful monitoring of its serum concentration, when used by patients with liver disease to optimize the dose[2].

Several methods have been reported for the determination of RIF in body fluids and include High Performance Liquid Chromatography (HPLC)[3,4], cyclic and square wave voltammetry[5], fluorimetry and microbiology[6], nuclear magnetic resonance spectrometry[7], chemometrics-aided kinetic spectrophotometry[8] and visible spectrophotometry[9-15]. In combination with other antituberculosis drugs such as isoniazid, pyrazinamide and ethambutol, RIF in pharmaceutical dosage form has been assayed by HPLC[16,17], HPTLC[18,19], linear sweep and cyclic voltammetry[20], cyclic and square wave voltammetry[5], differential pulse polarography[21,22] and chemiluminescence spectrometry[23,24]. The RIF, when present alone in capsules was quantitated by HPLC[25], differential pulse polarography[26,27], horseradish peroxidase-based amperometry[28], chemiluminescence spectrometry[29], nuclear magnetic resonance spectrometry[7] and visible spectrophotometry[9-15, 30-34].

The demand for fast and reliable measurement of RIF has necessiated the need for simple, easy to handle and inexpensive method. UV-spectrophotometry with its variants constitutes an evident alternative to the already existing methods. Multivariation calibration based on partial least square methods[35-37] were presented for the simultaneous determination of RIF, isoniazid (INH) and pyrazinamide (PYR)[35,36] and RIF, INH, PYR and ethambutol[37]. Benetton *et al.*[38] described first derivative UV-spectrophotometric method for RIF and INH whereas the same method was employed by Rote and Sharma[39] for the determination of RIF in combination with INH and PYR. A method based on the convolution of the double divisor ratio spectra was employed by Youssef and Maher[40] for resolving and assaying a ternary mixture containing RIF, INH and PYR. Three methods[41] based on the measurement of graphical absorbance ratio, derivative ratio and additivity of absorbances were developed for the simultaneous determination of RIF and INH. However, there is only one report on the application of direct UV-spectrophotometry[9] for the assay of RIF in formulations in which absorbance of an aqueous solution was measured at 340 nm. However, this method is not stability-indicating.

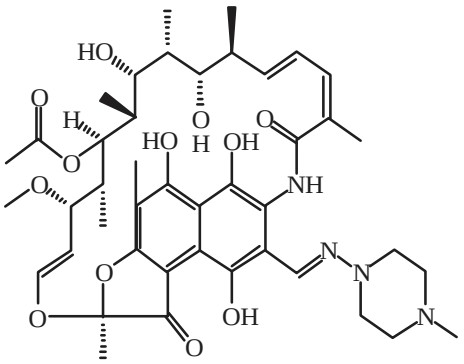

Fig. 1: Chemical structure of rifampicin

The need for a simple, sensitive, economical and faster analytical method in pharmaceutical quality control and clinical laboratories need not be overemphasized. Two UV-spectrophotometric methods which are stability-indicating were developed and validated for the determination of rifampicin in bulk drug, capsules and spiked human urine.

The stability-indicating assay is a method that is employed for the analysis of stability of samples in pharmaceutical industry. With the advent of ICH guidelines[42,43], the requirement of establishment of Stability Indicating Assay Method (SIAM) has become more clearly mandated.

The guidelines explicitly require conduct of forced degradation studies under a variety of conditions like pH, light, oxidation, dry heat, etc. and separation of drug and degradation products. A review on the development of validated Stability Indicating Assay Methods (SIAMs) for drug substances and products is available[44].

In this study, we developed and evaluated the performance of two simple, rapid and validated UV-spectrophotometric methods for RIF in pharmaceuticals and urine, which are stability-indicating. The methods are based on the measurement of the native absorbances of RIF in 0.1 M HCl (method A) and 0.1 M H_3PO_4 (method B). The methods were demonstrated to be sensitive, selective, accurate and precise, besides being robust and rugged.

MATERIALS AND METHODS

Materials and reagents: Pharmaceutical grade RIF (99.9% purity) was a gift from Lupin Limited, Tarapur, Maharashtra, India and was used as received. Capsules in two strengths R-Cin 300 and R-Cin 450 capsules (Lupin Limited, Chikaltana, Aurangabad, India) were purchased from local commercial stores. Solvents such as chloroform (99.4% alcohol

stabilized and spectroscopic grade), sodium hydroxide, potassium hydrogen orthophosphate, hydrochloric acid, orthophosphoric acid and hydrogen peroxide were purchased from Merck, Mumbai, India. Double distilled water was used throughout the investigation.

Sodium hydroxide solution (NaOH, 5 M) was prepared by dissolving required amount of pellets in water. Hydrochloric acid (HCl, 5 M) was prepared by appropriate dilution of concentrated acid (Specific gravity 1.18) with water. This solution was diluted to 0.1 M and standardized[45]. Orthophosphoric acid (0.1 M) was prepared by appropriate dilution of concentrated acid (Specific gravity 1.57) with water. About 3% solution of H_2O_2 was prepared by diluting required volume of the commercially available 30% reagent with water. A phosphate buffer of pH 7.4 was prepared by mixing 50 mL of 0.1 M potassium hydrogen phosphate and 25 mL of 0.1 M NaOH, diluted to 100 mL with water and pH adjusted using pH meter. Human urine was collected from a healthy male aged about 30 years.

Preparation of standard drug solution: Two stock standard solutions of 100 μg mL^{-1} RIF both were prepared by dissolving 10 mg each of pure RIF separately in 0.1 M HCl (method A) and 0.1 M H_3PO_4 (method B) and diluted separately to 100 mL with respective solvents in calibrated flasks.

Procedures for calibration curve

Method A (Using 0.1 M HCl): Into a series of 10 mL calibrated flasks, aliquots of standard drug solution (0.15-3.0 mL of 100 μg mL^{-1}) equivalent to 1.5-30 μg mL^{-1} RIF were accurately transferred and the volume was made up to the mark with 0.1 M HCl. The absorbance of each solution was then measured at 263 nm against 0.1 M HCl as the blank.

Method B (Using 0.1 M H_3PO_4): Aliquots (0.0, 0.15, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL) of RIF standard solution (100 μg mL^{-1}) were accurately measured into a series of 10 mL graduated flasks and the volume was made up to the mark with 0.1 M H_3PO_4 and the absorbance of each solution was measured at 259 nm against 0.1 M H_3PO_4 as reagent blank.

A calibration curve was prepared in both methods by plotting the absorbance versus concentration of drug. The concentration of the unknown was computed from the respective regression equation derived using the Beer's law data in both cases.

Preparation of capsule extract and assay procedure: Contents of 20 capsules were pooled and pulverized. The amount of capsule powder equivalent to 10 mg RIF was quantitatively transferred into two separate 100 mL volumetric flasks. The content in each flask was shaken well with about 50 mL of 0.1 M HCl or 0.1 M H_3PO_4 separately for 20 min and the content was diluted to the mark with the respective solvent. It was filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and 2 mL portion of the subsequent portion was subjected to analysis following the general procedures described earlier.

Procedure for analysis of spiked human urine: To prepare spiked urine sample[7], 10 mg of the pure RIF and 10 mL of urine sample were transferred into a separating funnel, mixed well till dissolution was complete. The solution was extracted with three 10 mL portion of chloroform and the organic layer was collected in a beaker after drying over anhydrous sodium sulphate. The solvent was evaporated to dryness. The resulting residue was reconstituted with either 0.1 M HCl and diluted to 100 mL or with 0.1 M H_3PO_4 before diluting to 100 mL with the same solvent. Then, the analysis was performed in replicates as described under general procedure for pure RIF by taking 2 mL of the resulting urine solution.

Forced degradation studies: A stock solution containing 10 mg of RIF either in 100 mL of 0.1 M HCl or in 100 mL of 0.1 M H_3PO_4 was prepared separately. This stock solution (100 μg mL^{-1}) was used for forced degradation under acid, base and peroxide-induced stress conditions to provide an indication of the stability-indicating property of the methods. About 1.5 mL of this solution was accurately transferred to separate 10 mL volumetric flasks. Three milliliters each of 5 M HCl, 5 M NaOH or 3% H_2O_2 were added to the flasks separately and the flasks were heated for 3 h on a water bath maintained at 80°C. Then the solutions were cooled and neutralized by adding base or acid, the volume in each flask was brought to the mark with 0.1 M HCl or 0.1 M H_3PO_4 separately and absorbance measured at 263 nm in method A and 259 nm in method B. Solid state thermal degradation was carried out by exposing pure drug to dry heat at 105°C for 24 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber for 24 h. The sample after exposure to heat and light was used to prepare 100 μg mL^{-1} solutions in 0.1 M HCl and 0.1 M H_3PO_4 separately and the absorbance measured at 263 nm versus 0.1 M HCl in method A and 259 nm versus 0.1 M H_3PO_4 in method B.

Linearity, limits of detection and quantification: Three series (analytical curves) of standard solutions of RIF were prepared separately by the dilution of the stock standard solution in

0.1 M HCl and 0.1 M H_3PO_4 and absorbances were measured, in triplicate, at 263 nm in method A and 259 nm in method B, respectively. The limits of detection (LOD) and quantification (LOQ) were calculated directly from the calibration plot using the formulae:

$$LOD = 3.3S/b$$

$$LOQ = 10S/b$$

where, S is the standard deviation of blank absorbance values and b is the slope of the calibration plot[43].

Within-day and day to day accuracy and precision: The within-day and day to day precisions of the proposed methods were evaluated by measuring the absorbance 7 times on the same day and on five different days using three different concentrations of RIF (10, 20 and 25 μg mL^{-1}). From the absorbance values obtained, concentration was calculated and the results were expressed as percentage Relative Standard Deviation (RSD%). The accuracy was evaluated as percentage Relative Error (RE%) between the found and taken concentrations.

Accuracy: The accuracy and validity of the proposed methods were evaluated by performing recovery studies. Pre-analyzed tablet powder was spiked with pure RIF at three concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. The recovery (%) of the pure drug added was calculated as:

$$\text{Recovery (\%)} = [(C_i-C_a)/C_s]\times 100$$

where, C_i is the total drug concentration measured after standard addition, C_s is drug concentration in the formulation sample and C_a is drug concentration added to the formulation[42].

Robustness and ruggedness: Robustness of the proposed methods was determined by the analysis of samples and standard solutions (10, 20 and 25 μg mL^{-1}) at different wavelengths. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from instrument to instrument and from analyst to analyst. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by three analysts using same operational and environmental conditions and by a single analyst with three different cuvettes.

Selectivity by placebo and synthetic mixture analyses: A placebo blank of the composition Barsoum *et al.*[9]: Urea (10 mg), sodium oxalate (15 mg), camphor (10 mg), glucose (10 mg), lactose (20 mg), sucrose (15 mg) and ascorbic acid (10 mg) was made and its solution was prepared as described 'procedure for capsules' and then subjected to analysis.

To assess the role of the inactive ingredients on the assay of RIF, a synthetic mixture was separately prepared by adding 10 mg of RIF to the 10 mg placebo. The drug was extracted and solution was prepared as described under the 'procedure for capsules'. The solutions after appropriate dilution were analyzed following the recommended procedures.

RESULTS AND DISCUSSION

The aim of this study was to validate two simple, rapid and eco-friendly methods to assay RIF in formulations by UV-spectrophotometry. In method A 0.1 M HCl was used as the solvent and the absorbance was measured at 263 nm (Fig. 2a) where as in method B, 0.1 M H_3PO_4 was used as a solvent with the measurement being made at 259 nm analytical wavelength (Fig. 2b).

Validation protocol

Linearity: A linear relationship was found between the absorbances at 263 nm in method A and 259 nm in method B and the concentration of RIF in the range 1.5-30 μg mL^{-1} in both methods as shown in Fig. 3. The correlation coefficients (r) were 0.9995 and 0.9997 indicating good linearity. The representative linear equations were $y = 0.0303x+0.0008$ and $y = 0.0316x+0.0046$ calculated by the respective least squares method, for method A and method B, respectively. Optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values were calculated. The limits of detection (LOD) and quantification (LOQ) were also calculated and all these data are presented in Table 1. The uncertainties with the y-axis (S_y), intercept (S_a) and slope (S_b) were also calculated for both methods. These results are presented in Table 1.

Precision and accuracy: The results of within-day and day to day analysis of the sample are given in Table 2. As evident, RSD% values of the data obtained were all below 3% (i.e., in the range of 1.67-2.72 and 1.58-2.29% for within-day and day to day, respectively). The RSD% values indicated that the proposed methods are sufficiently precise.

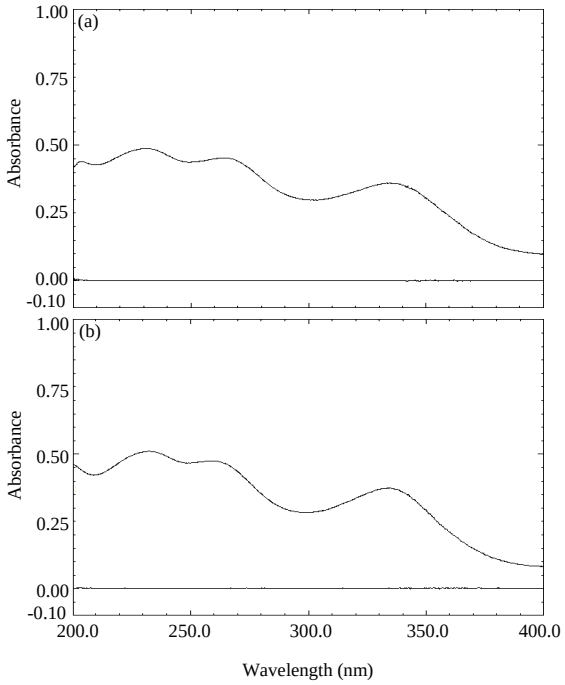

Fig. 2(a-b): UV absorption spectra of (a) RIF (15 μg mL^{-1}) in 0.1 M HCl and (b) RIF (15 μg mL^{-1}) in 0.1 M H_3PO_4

Table 1: Sensitivity and regression parameters

	Methods	
Parameters	A	B
λ_{max} (nm)	263	259
Linear range (μg mL^{-1})	1.5-30	1.5-30
Molar absorptivity (ε) (L mol^{-1} cm^{-1})	2.49×10^4	2.71×10^4
Sandell sensitivity* (μg cm^{-2})	0.0330	0.0304
Limit of detection (LOD) (μg mL^{-1})	0.19	0.14
Limit of quantification (LOQ) (μg mL^{-1})	0.57	0.44
Intercept (a)	0.0008	0.0046
Slope (b)	0.0303	0.0316
S_a	0.0998	0.0998
S_b	3.89×10^{-3}	3.89×10^{-3}
Regression coefficient (r)	0.9995	0.9997

*Limit of determination as the weight in μg mL^{-1} of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm, Y = a+bX, where, Y is the absorbance, X is concentration in μg mL^{-1}, a is intercept and b is slope, S_y or S_a is standard deviation of intercept and S_b is standard deviation of slope

Table 2: Evaluation of intra-day and inter-day accuracy and precision

		Intra-day accuracy and precision (n = 7)			Inter-day accuracy and precision (n = 7)		
Methods	RIF taken (μg mL^{-1})	RIF found[a] (μg mL^{-1})	RSD[b] (%)	RE[c] (%)	RIF found[a] (μg mL^{-1})	RSD[b] (%)	RE[c] (%)
A	10	10.12	1.02	1.20	10.16	1.19	1.60
	20	20.15	1.37	0.75	20.21	0.87	1.05
	25	24.74	0.98	1.04	24.65	1.24	1.36
B	15	14.79	1.62	1.40	14.72	1.01	1.87
	20	19.69	0.97	1.55	19.59	0.89	2.05
	25	25.21	1.14	0.84	25.36	1.52	1.44

[a]Mean value of 7 determinations, [b]Relative standard deviation (%), [c]Relative error (%)

As shown from the data presented in Table 2, the relative error between the taken and found concentrations of RIF is <2.05% indicating that the proposed methods are quite accurate for the assay of RIF.

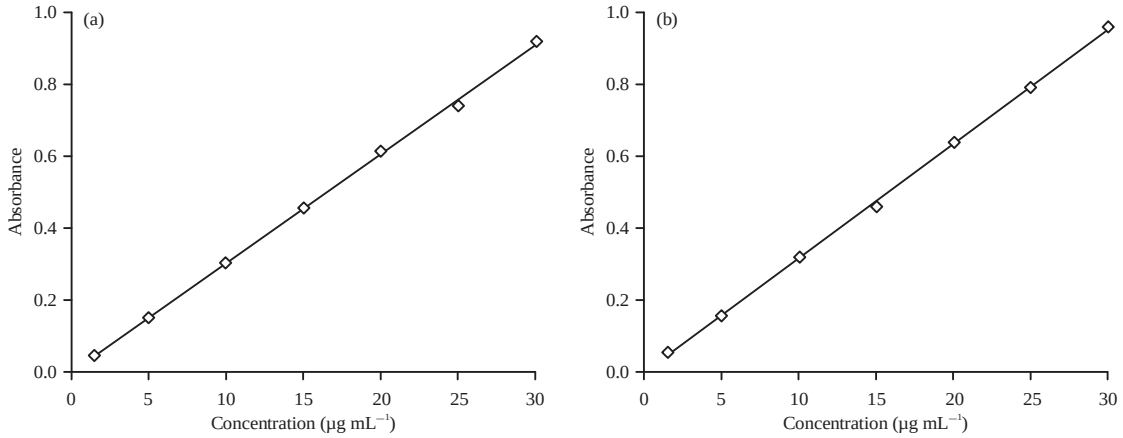

Fig. 3(a-b): Calibration plot for (a) Method A and (b) Method B

Table 3: Results of robustness and ruggedness expressed as intermediate precision

		Robustness	Ruggedness	
Methods	RIF taken (µg mL^{-1})	Parameter altered wavelength* (RSD%) (n = 3)	Inter-analysis (RSD%) (n = 3)	Inter-cuvettes (RSD%) (n = 3)
A	10	2.14	0.88	1.45
	20	3.02	1.54	0.93
	25	2.63	0.91	1.14
B	15	2.59	0.54	1.09
	20	2.02	2.14	0.63
	25	2.07	0.72	0.91

*Wavelengths used were 262, 263 and 264 nm in method A and 258, 259, 260 nm in method B

Table 4: Results of analysis of capsules by the proposed methods and statistical comparison of the results with the reference method

		Found *(Percent of label claim±SD)		
			Proposed methods	
Capsules brand name	Nominal amount (mg tablet^{-1})	Reference method	A	B
R-Cin 300	300	98.27±1.29	99.15±0.72	99.69±1.12
			F = 3.21	F = 1.33
			t = 1.34	t = 1.86
R-Cin 450	450	101.4±0.91	99.35±1.45	102.1±1.72
			F = 2.54	F = 1.51
			t = 2.63	t = 2.59

*Mean value of five determinations, tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77, tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39

Robustness and ruggedness: In addition, the reliability of the proposed methods was also evaluated by means of robustness test. The absorbances of standard and sample solutions were determined at the UV wavelength used in this study λ_{max}±1 nm. No significant difference was observed in the results found. Intermediate precision values (RSD%) were in the range 0.54-2.63% indicating acceptable robustness. Percent RSD values obtained as a part of the ruggedness study by recording the absorbance values using three cuvettes by a single analyst and by three analysts with a single cuvette are also presented in Table 3.

Application to capsule analysis: Commercial RIF capsules were analyzed using the proposed methods and also by a reference method[38]. Capsule extract equivalent to 100 µg mL^{-1} RIF was prepared in methanol and 5 mL of this extract was diluted to 10 mL with phosphate buffer of pH 7.4 and absorbance measured at 475 nm vs buffer. The results obtained were compared statistically by the Student's t-test and the variance-ratio F-test. The calculated t- and F-values did not exceed the tabulated values of 2.77 and 6.39 at the 95% confidence level and for four degrees of freedom (Table 4), indicating close similarity

between the proposed method and the reference method with respect to accuracy and precision.

Recovery study: To further ascertain the accuracy and reliability of the proposed methods, recovery experiments were performed via standard-addition technique. Pre-analyzed capsule powder was spiked with pure RIF at three different levels and the total was found by the proposed methods. Each determination was repeated 3 times. The percent recovery of pure RIF added was within the permissible limits indicating the absence of inactive ingredients in the assay. These results are as illustrated in Table 5.

Application to spiked human urine: The proposed methods were applied to human urine by taking 20 μg mL^{-1} extracted urine solution in replicates (n = 5). The recoveries obtained is 91.74% with standard deviation 0.91 for method A and 92.16 with 0.57 for method B indicated the accessibility of the methods and the results are given in Table 6.

Selectivity: The absorbance of the placebo solution in each case was almost equal to the absorbance of the blank which revealed no interference. The absorbance resulting from 20 μg mL^{-1} (in both methods) was nearly the same as those obtained for pure RIF solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of RIF.

Stability-indicating property: The RIF was subjected to acid, base and hydrogen peroxide induced degradation in solution state and photo and thermal degradation in solid state. The study was performed by measuring the absorbance of RIF solution only after subjecting to forced degradation. From the response, percentage recovery of RIF was calculated in each case and is presented in Table 7. Degradation study showed that very slight degradation was observed under peroxide-slight degradation under acid and substantial degradation under-base induced degradation in both methods. No degradation was observed under other stress conditions (Fig. 4-8).

Table 5: Results of recovery experiment via standard-addition procedure

Methods	Capsules brand name	RIF in capsules (μg mL^{-1})	Pure RIF added (μg mL^{-1})	Total found (μg mL^{-1})	Pure RIF recovered (Percent±SD*)
A	R-Cin 300	9.92	5.0	15.26	102.3±1.03
		9.92	10.0	19.70	98.9±0.98
		9.92	15.0	25.34	101.7±1.12
	R-Cin 450	9.97	5.0	15.16	101.3±0.72
		9.97	10.0	29.82	99.5±0.89
		9.97	15.0	25.12	100.6±1.05
B	R-Cin 300	9.94	5.0	14.69	98.3±1.63
		9.94	10.0	20.44	102.5±1.89
		9.94	15.0	25.24	101.2±0.91
	R-Cin 450	10.21	5.0	15.41	101.3±1.23
		10.21	10.0	19.95	98.7±0.55
		10.21	15.0	25.01	99.2±1.19

*Mean value of three determinations

Table 6: RIF determination in spiked urine sample (n = 5)

Methods	Spiked concentration (μg mL^{-1})	Concentration found* (μg mL^{-1})	Percentage of recovery±SD*
A	20	18.35	91.74±0.91
B	20	18.43	92.16±0.57

*Mean value of five determinations of RIF

Table 7: Results of forced degradation studies

		RIF found* (μg mL^{-1})		Recovery percentage of RIF±SD	
		----------(Methods)----------		----------(Methods)----------	
Parameters studied	RIF taken (μg mL^{-1})	A	B	A	B
Acid hydrolysis	15.0	9.56	9.80	63.7±1.02	65.3±0.72
Alkaline hydrolysis	15.0	6.27	5.79	41.8±0.81	38.6±0.54
Neutral hydrolysis	15.0	15.02	15.04	100.1±0.78	100.3±0.69
Oxidative degradation	15.0	13.40	13.38	89.3±0.61	89.2±0.98
Thermal degradation	15.0	14.98	15.02	99.9±0.58	100.1±0.68
Photo degradation	15.0	15.02	14.76	100.1±0.97	98.4±0.51

*Mean value of three determinations

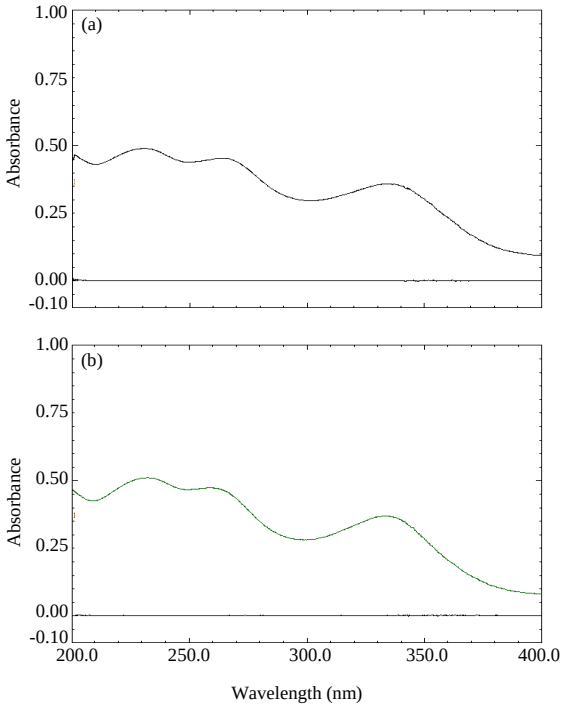

Fig. 4(a-b): UV absorption spectra of thermal degraded product (a) RIF (15 µg mL^{-1}) at 259 nm in 0.1 M HCl and (b) RIF (15 µg mL^{-1}) in 0.1 M H_3PO_4

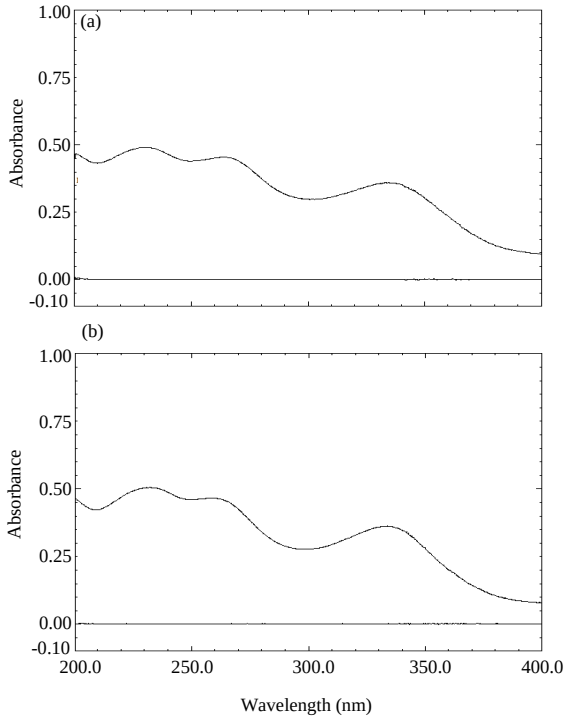

Fig. 5(a-b): UV absorption spectra of photolytic degraded product (a) RIF (15 µg mL^{-1}) at 259 nm in 0.1 M HCl and (b) RIF (15 µg mL^{-1}) in 0.1 M H_3PO_4

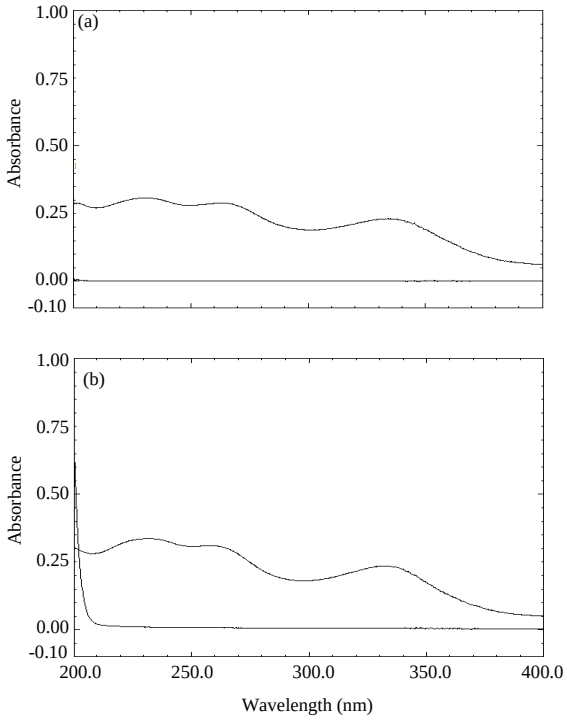

Fig. 6(a-b): UV absorption spectra of acid degraded product (a) RIF (15 µg mL^{-1}) at 259 nm in 0.1 M HCl and (b) RIF (15 µg mL^{-1}) in 0.1 M H_3PO_4

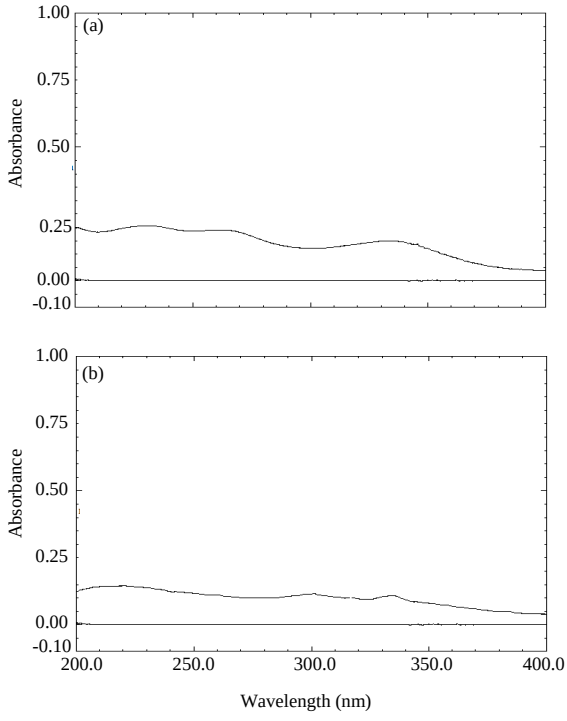

Fig. 7(a-b): UV absorption spectra of base degraded product (a) RIF (15 µg mL^{-1}) at 259 nm in 0.1 M HCl and (b) RIF (15 µg mL^{-1}) in 0.1 M H_3PO_4

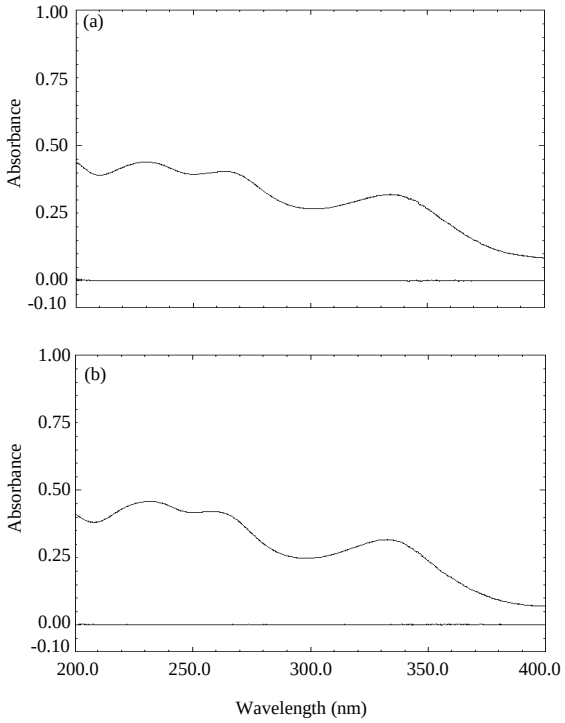

Fig. 8(a-b): UV absorption spectra of oxidative degraded product (a) RIF (15 µg mL^{-1}) at 259 nm in 0.1 M HCl and (b) RIF (15 µg mL^{-1}) in 0.1 M H_3PO_4

CONCLUSION

Two simple, rapid and sensitive methods are reported for the determination of rifampicin in the bulk form, capsules and spiked-human urine. This is the first study on the stability-indicating methods for rifampicin. The methods presented here are highly sensitive and rapid and require no organic solvents or any additional reagents. Further the methods are free from any tedious procedural or extraction steps. The instrument employed is cheap, easy to handle and no expertise personnel is required. They can be considered to be a promising alternative to HPLC. The proposed methods show clear advantages, such as short analysis time and no pretreatment or time-consuming extraction step (except for urine) were required prior to analysis. Moreover, because of its low limits of detection and quantification, the methods could be applied in clinical laboratories and pharmacokinetic studies.

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REFERENCES

1. Barnes, P.F. and S.A. Barrows, 1993. Tuberculosis in the 1990s. Ann. Intern. Med., 119: 400-410.
2. Oldfield, S., J.D. Berg, H.J. Stiles and B.M. Buckley, 1986. Measurement of rifampicin and 25-desacetylrifampicin in biological fluids using high-performance liquid chromatography with direct sample injection. J. Chromatogr. B: Biomed. Sci. Applic., 377: 423-429.
3. Khuhawar, M.Y. and F.M.A. Rind, 2002. Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood. J. Chromatogr. B, 766: 357-363.
4. Walubo, A., P. Smith and P.I. Folb, 1994. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography. J. Chromatogr. B: Biomed. Sci. Applic., 658: 391-396.
5. Hammam, E., A.M. Beltagi and M.M. Ghoneim, 2004. Voltammetric assay of rifampicin and isoniazid drugs, separately and combined in bulk, pharmaceutical formulations and human serum at a carbon paste electrode. Microchem. J., 77: 53-62.

6. Finkel, J.M., R.F. Pittillo and L.B. Mellett, 1971. Fluorometric and microbiological assays for rifampicin and the determination of serum levels in the dog. Chemotherapy, 16: 380-388.
7. Salem, A.A., H.A. Mossab and B.N. Barsoum, 2005. Quantitative determinations of levofloxacin and rifampicin in pharmaceutical and urine samples using nuclear magnetic resonance spectroscopy. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc., 62: 466-472.
8. Wang, Y., Y. Ni and S. Kokot, 2008. Simultaneous kinetic spectrophotometric determination of norfloxacin and rifampicin in pharmaceutical formulation and human urine samples by use of chemometrics approaches. Sci. China Ser. B: Chem., 51: 776-785.
9. Barsoum, N.B., M.S. Kamel and M.M.A. Diab, 2008. Spectrophotometric determination of isoniazid and rifampicin from pharmaceutical preparations and biological fluids. Res. J. Agric. Biol. Sci., 4: 471-484.
10. Gandhi, T.P., A.A. Patel, P.R. Patel and V.C. Patel, 1978. Colorimetric estimation of rifampicins in formulations and biological fluids by metallic ions. Indian Drugs, 16: 10-12.
11. Reddy, B.S. and C.S.P. Sastry, 1983. Ion-pair extraction method for ethambutol, ethionamide and rifampicin determination. J. Inst. Chem. (India), 55: 69-70.
12. Sadeghi, S. and E. Karimi, 2006. Spectrophotometric determination of rifampicin through chelate formation and charge transfer complexation in pharmaceutical preparation and biological fluids. Chem. Pharm. Bull., 54: 1107-1112.
13. Sastry, C.S.P., T.E. Divakar and U.V. Prasad, 1985. Spectrophotometric determination of rifampicin with some metal ions. Indian Drugs, 22: 604-606.
14. Sastry, C.S.P., T.E. Divakar and U.V. Prasad, 1985. Spectrophotometric determination of rifampicin with chloranil. Indian J. Pharm. Sci., 47: 45-46.
15. Sastry, C.S.P., T.E. Divakar and U.V. Prasad, 1986. Spectrophotometric determination of rifampicin with uranyl or thorium(IV). J. Instt. Chem. (India), 58: 17-18.
16. Calleri, E., E. de Lorenzi, S. Furlanetto, G. Massolini and G. Caccialanza, 2002. Validation of a RP-LC method for the simultaneous determination of isoniazid, pyrazinamide and rifampicin in a pharmaceutical formulation. J. Pharm. Biomed. Anal., 29: 1089-1096.
17. Gunasekaran, S. and E. Sailatha, 2009. Estimation of pyrazinamide, isoniazid and rifampicin in pharmaceutical formulations by high performance liquid chromatography method. Asian J. Chem., 21: 3561-3566.
18. Ali, J., N. Ali, Y. Sultana, S. Baboota and S. Faiyaz, 2007. Development and validation of a stability-indicating HPTLC method for analysis of antitubercular drugs. Acta Chromatogr., 18: 168-179.
19. Shewiyo, D.H., E. Kaale, P.G. Risha, B. Dejaegher, J. Smeyers-Verbeke and Y. Vander Heyden, 2012. Optimization of a reversed-phase-high-performance thin-layer chromatography method for the separation of isoniazid, ethambutol, rifampicin and pyrazinamide in fixed-dose combination antituberculosis tablets. J. Chromatogr. A, 1260: 232-238.
20. Wahdan, T., 2005. Voltammetric method for the simultaneous determination of rifampicin and isoniazid in pharmaceutical formulations. Chem. Anal., 50: 457-464.
21. Lomillo, M.A.A., O.D. Renedo and M.J.A. Martinez, 2001. Resolution of ternary mixtures of rifampicin, isoniazid and pyrazinamide by differential pulse polarography and partial least squares method. Anal. Chim. Acta, 449: 167-177.
22. Asadpour-Zeynali, K. and P. Soheili-Azad, 2010. Simultaneous polarographic determination of isoniazid and rifampicin by differential pulse polarography method and support vector regression. Electrochim. Acta, 55: 6570-6576.
23. Li, B., Y. He, J. Lv and Z. Zhang, 2005. Simultaneous determination of rifampicin and isoniazid by continuous-flow chemiluminescence with artificial neural netstudy calibration. Anal. Bioanal. Chem., 383: 817-824.
24. Halvatzis, S.A., M.M. Timotheou-Potamia and T.P. Hadjiioannou, 1993. Continuous-flow chemiluminometric determination of dihydralazine, rifampicin and rifamycin SV by oxidation with N-bromosuccinimide. Anal. Chim. Acta, 272: 251-263.
25. Shah, Y., S. Khanna, K.C. Jindal and V.S. Dighe, 1992. Determination of rifampicin and isoniazid in pharmaceutical formulations by HPLC. Drug Dev. Ind. Pharm., 18: 1589-1596.
26. Lomillo, M.A.A., O.D. Renedo and M.J.A. Martinez, 2002. Optimization procedure, applying the experimental-design methodology, for the determination of rifampicin after metal complexation by differential pulse adsorptive stripping voltammetry. Helvet. Chim. Acta, 85: 2430-2439.
27. Hahn, Y. and S. Shin, 2001. Electrochemical behavior and differential pulse polarographic determination of rifampicin in the pharmaceutical preparations. Arch. Pharma. Res., 24: 100-104.
28. Lomillo, M.A.A., J.M. Kauffmann and M.J.A. Martinez, 2003. HRP-based biosensor for monitoring rifampicin. Biosens. Bioelectron., 18: 1165-1171.
29. Ma, Y., B.T. Zhang, L.X. Zhao, G.S. Guo and J.M. Lin, 2008. Determination of rifampicin by peroxomonosulfate-cobalt(II) chemiluminescence system. Chin. J. Chem., 26: 905-910.
30. Divakar, T.E., U.V. Prasad and C.S.P. Sastry, 1985. Spectrophotometric estimation of tetracyclines and rifampicin using p-N, N-dimethylphenylenediamine and chloramine T. Indian Drugs, 22: 328-329.
31. Divakar, T.E., S. Sunitha, G.K. Deepthi, T. Benjamin and N.P. Babu, 2012. Assay of rifampicin in bulk and its dosage forms by visible spectrophotometry using chloranilic acid. Int. J. Chem. Environ. Pharm. Res., 3: 64-67.

32. Rao, G.R., S.S.N. Murty and E.V. Rao, 1985. Spectrophotometric determination of rifampicin in pharmaceutical dosage forms. Indian Drugs, 22: 484-488.
33. Galal, S.M., S.M. Blaih and M.E. Abdel-Hamid, 1992. Comparative spectrophotometrc analysis of rifampicin by chelate formation and charge-transfer complexation. Anal. Lett., 25: 725-743.
34. Shukla, I.C., P.K. Dwivedi, S. Kumar, B.K. Singh and A. Dubey, 2007. Application of ammonium metavanadate for the determination of some tubercular and adrenocortical steroid drugs. J. Indian Chem. Soc., 84: 100-102.
35. Espinosa-Mansilla, A., M.A. Valenzuela, A.M. de la Pena, F. Salinas and F.C. Canada, 2001. Comparative study of partial least squares and a modification of hybrid linear analysis calibration in the simultaneous spectrophotometric determination of rifampicin, pyrazinamide and isoniazid. Anal. Chim. Acta, 427: 129-136.
36. Goicoechea, H.C. and A.C. Olivieri, 1999. Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. J. Pharm. Biomed. Anal., 20: 681-686.
37. Marcellos, L.F., A.F. Faria, M.V.N. de Souza, M.R. Almeida, G.P. Sabin, R.J. Poppi and M.A.L. de Oliveira, 2012. Simultaneous analysis of first-line anti-tuberculosis drugs in tablets by UV spectrophotometry compared to capillary zone electrophoresis. Central Eur. J. Chem., 10: 1808-1816.
38. Benetton, S.A., E.R.M. Kedor-Hackmann, M.I.R.M. Santoro and V.M. Borges, 1998. Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations. Talanta, 47: 639-643.
39. Rote, A.R. and A.K. Sharma, 1997. Simultaneous spectrophotometric determination of rifampicin, isoniazid and pyrazinamide by first-derivative UV spectrophotometry in combined pharmaceutical dosage forms. Indian J. Pharm. Sci., 59: 119-123.
40. Youssef, R.M. and H.M. Maher, 2008. A new hybrid double divisor ratio spectra method for the analysis of ternary mixtures. Spectrochimica Acta Part A: Mol. Biomol. Spectrosc., 70: 1152-1166.
41. Kakde, R.B., A.V. Kasture and S.G. Wadodkar, 2002. Spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations. Indian J. Pharm. Sci., 64: 24-27.
42. ICH., 2005. Validation of analytical procedures: Methodology (Q2AR1). Proceedings of the International Conference on Harmonization, November 1991 and November 2005, Food and Drug Administration, USA.
43. ICH., 2013. Stability testing of new drug substances and products (Q1AR2). Proceedings of the International Conference on Harmonization, November 1996 and February 2013, Food and Drug Administration, USA.
44. Bakshi, M. and S. Singh, 2002. Development of validated stability-indicating assay methods-critical review. J. Pharm. Biomed. Anal., 28: 1011-1040.
45. Vogel, A.I., 1961. A Text-Book of Quantitative Inorganic Analysis. 3rd Edn., The English Language Book Edition Society, London.