Hindawi Publishing Corporation
ISRN Analytical Chemistry
Volume 2013, Article ID 613218, 9 pages
http://dx.doi.org/10.1155/2013/613218

Research Article

Stability Indicating HPTLC Method for Analysis of Rifaximin in Pharmaceutical Formulations and an Application to Acidic Degradation Kinetic Study

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Received 16 June 2013; Accepted 26 July 2013

Academic Editors: Z. Aydogmus, E. Billiot, E. Lodyga-Chruscinska, and J. J. Santana-Rodríguez

A specific stability indicating high-performance thin-layer chromatographic method for analysis of rifaximin both as a bulk drug and in formulations was developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The optimized mobile phase system consisted of n-hexane : 2-propanol : acetone : ammonia (5 : 4.1 : 1, v/v/v/v), which gave compact spots for rifaximin at R_f of 0.59 ± 0.03. Rifaximin was subjected to forced degradation studies in order to check the specificity of the method. Densitometric analysis of rifaximin was carried out in the absorbance reflectance mode at 443 nm. The calibration plots showed linear relationship in the concentration range of 400–3200 ng per band. Moreover, linearity was also confirmed by verification of homoscedasticity of variance. According to validation studies, the developed method was repeatable and specific as revealed by % RSD less than 2 and hence can be used for routine analysis of pharmaceutical formulation. Moreover, the method could effectively separate the drug from its degradation products; hence it can be employed as a stability indicating one. The kinetics of acid degradation process at various temperatures was also investigated and first-order rate constant, half-life, shelf life, and activation energy were computed.

1. Introduction

Rifaximin, a benzimidazole derivative, is a structural analogue of rifampicin. Chemically, it is a 2S,16Z,18E,20S,21S, 22R,23R,24R,25S,26S,27S,28E-5,6,21,23,25-pentahydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-2,7-epoxypentadeca-[1,11,13] trienimino) benzofuro [4,5-e] pyrido [1,2 benzimidazole 1,15(2H)-dione,25-acetate (Figure 1) [1].

Rifaximin is a newer antibiotic, used for the treatment of patients having more than 12 years of age with traveller's diarrhoea caused by noninvasive strains of *Escherichia coli* [4]. Rifaximin binds to the beta-subunit of bacterial DNA-dependent RNA polymerase and prevents catalysis of polymerization of deoxyribonucleotides into a DNA strand, thereby inhibiting bacterial RNA synthesis. In vitro studies of rifaximin have demonstrated broad-spectrum coverage including gram-positive, gram-negative, and anaerobic bacteria as well as a limited risk of bacterial resistance [5].

Literature reports various analytical methods like spectrophotometric [6, 7], RP-HPLC [8, 9], and stability indicating HPLC [10] for the determination of rifaximin in pharmaceutical formulations. Moreover, bioanalytical methods, that is, HPLC-TMS [11, 12], LC-ESI-MS method for determination of rifaximin in human plasma, dried blood spot [2], are also reported in literature. However, none of the articles related to the stability indicating high-performance thin-layer chromatographic (HPTLC) method for the determination of rifaximin in pharmaceutical formulation has ever been mentioned in literature up to our knowledge.

An ideal stability indicating analytical method is one that quantifies the drug and also resolves its degradation products. The ICH guideline explicitly states the requirement

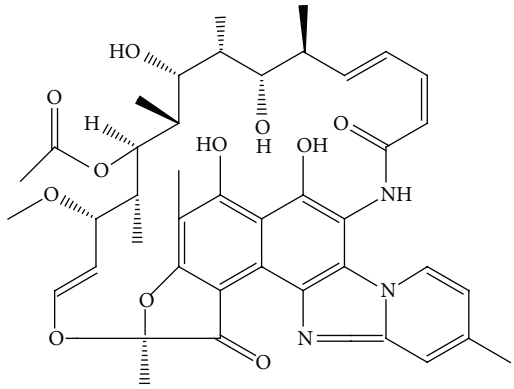

Figure 1: Chemical structure of rifaximin.

of stability indicating methodology by conducting forced degradation studies under a variety of conditions, like pH, light, oxidation, dry heat, and so forth, and for separating drug from its degradation products, [3, 13]. Moreover, forced degradation studies are carried out to establish the inherent stability characteristics and degradation pathways under various conditions [14]. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf lives to be established [15].

Although stability indicating methods have been reported for assay of various drugs in drug products, chromatographic methods have taken precedence over the conventional methods of analysis, showing greater accuracy and sensitivity for small amounts of degradation products generated [13, 16]. Nowadays, HPTLC as a rapid, accurate and fast method is becoming a routine analytical technique and stability-indicating methodology using HPTLC for different drugs has been reported in literature [16–18]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Unfortunately, HPTLC as an instrument is not routinely available is its major limitation [13].

The aim of the present work hence was to develop an economic, accurate, specific, and reproducible and stability-indicating HPTLC method for the determination of rifaximin in the presence of its degradation products and related impurities from pharmaceutical formulation. The proposed method was validated as per ICH guideline. Furthermore, the developed HPTLC method was also used to investigate the kinetics of the acidic degradation process by quantitation of drug at different temperatures and to calculate the activation energy and half-life for rifaximin degradation.

2. Experimental

2.1. Materials. Rifaximin was received as a gift sample from Lupin Pharma Ltd., Mumbai, India. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India. Marketed tablet formulations used in the study were RCIFAX 400 and 200 mg (Lupin Pharma Ltd.), RIFAGUT (Sunpharma) TORFIX (Torrent Pharma), and RIXMIN 200 mg (Cipla) procured from local market.

2.2. Instrumentation. Hamilton microlitre syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, Camag, Switzerland), precoated silica gel aluminium plate 60 F_{254}, (20 × 10 cm, 100 μm thickness; E. Merck, Darmstadt, Germany), Linomat 5 sample applicator (Camag, Switzerland), Twin trough chamber (20 × 10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 4 (Camag, Switzerland), and operated by winCATS version 1.4.6 software (Camag, Switzerland) were used in the study.

2.3. Chromatographic Development Procedure. Suitable volume of standard and sample solution was spotted in the form of bands having band width of 6 mm on precoated silica gel 60 F_{254} HPTLC plate, 8 mm from the bottom and 15 mm from the side edges in the form of bands. Linear ascending development was carried out and the optimized mobile phase consisted of n-hexane : 2-propanol : acetone : ammonia (5 : 4 : 1 : 1 v/v/v/v). The optimized chamber saturation time before chromatographic development was 20 min and the length of chromatographic run was 8 cm. Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed and all measurements were made in the reflectance absorbance mode at 443 nm, with slit dimension (6.00 × 0.30 mm, micro), scanning speed 20 mm/s, data resolution 100 μm/step, optical filter (second order), and filter factor (Savitsky golay 7). The source of radiation was deuterium lamp emitting a continuous UV spectrum between 200 and 700 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light and evaluation was done by ordinary linear regression analysis of peak area.

2.4. Calibration Curve. A standard stock solution was prepared by dissolving accurately weighed 10 mg of rifaximin in methanol in 10 mL volumetric flask, to obtain a concentration of 1000 μg/mL. Further dilution was done to obtain a working standard solution having concentration of 100 μg/mL. Different volumes of solution were applied to obtain a concentration range of 400–3200 ng/band of rifaximin, in five replicate measurements. The measured peak area versus corresponding concentration of rifaximin was evaluated by ordinary linear regression analysis.

2.5. Analysis of Tablet Formulation. To determine the amount of rifaximin in various tablet dosage forms (label claim: 400 and 200 mg per tablet), the contents of 20 tablets were weighed, their mean weight was determined, and they are finely powdered. An accurately weighed powder sample equivalent to 10 mg of rifaximin was transferred into a 100 mL volumetric flask containing 50 mL methanol, followed by sonication for 30 min and further dilution up to the mark

with methanol. The resulting solution was filtered through Whatman filter paper no. 42. Eight microlitres of the filtered solution (800 ng per band) was applied on the HPTLC plate followed by development and scanning as per optimized chromatographic conditions.

2.6. Method Validation. The method was validated in accordance with ICH guideline [19] for evaluation of the following method validation parameters.

2.6.1. Precision. Precision of the developed method was studied by performing repeatability and intermediate precision studies. Repeatability was carried out by performing three replicates of three different concentrations (800 ng, 1600 ng, and 3200 ng) and peak area measured was expressed in terms of percent relative standard deviation. The intermediate precision study was performed on different days. The intermediate precision was assessed by studying three different concentrations (800 ng, 1600 ng, and 3200 ng) for three different days.

2.6.2. Accuracy. Accuracy of method was ascertained by performing recovery at three concentration level of 50%, 100%, and 150%, by spiking rifaximin standard (500 ng, 1000 ng, and 1500 ng) to the dosage form (1000 ng/band). Recovery studies were performed in triplicate.

2.6.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ). As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the y-intercept and slope of the calibration curve of rifaximin using the formula

$$\begin{aligned} \text{LOD} &= 3.3 \times \frac{\sigma}{S}, \\ \text{LOQ} &= 10 \times \frac{\sigma}{S}, \end{aligned} \tag{1}$$

where "σ" is standard deviation of the response and "S" is slope of calibration curve.

2.6.4. Robustness. The effect of deliberate variations on method parameters like the composition of the mobile phase saturation time, development distance, spot scanning time interval, wavelength scan, time from spotting to chromatography, and mobile phase volume was evaluated. The effect of these changes on both the R_f values and peak areas was evaluated by calculating the % RSD for each parameter.

2.6.5. Specificity. The specificity of the method was ascertained by comparing both the chromatogram and spectra of samples of marketed formulation and degradation sample with standard drug. The spot for rifaximin in both samples was confirmed by comparing the R_f values in the chromatogram and overlaying peak purity spectra with of standard drug. The peak purity of rifaximin was assessed by comparing the spectra at three different levels, that is, peak start (S), peak apex (M), and peak end (E) position.

2.7. Forced Degradation Studies. To evaluate the stability indicating property of the developed HPTLC method, standard drug was subjected to acid/base hydrolysis, oxidation, wet heat, and photodegradation. 10 mg of accurately weighed rifaximin was transferred to 10 mL volumetric flask and diluted with methanol to obtain a final concentration of 1000 μg/mL. This solution was further subjected to following forced degradation study.

2.7.1. Acid-Induced Degradation Study. To 5 mL of the above standard drug solution, 5 mL of 0.1 M hydrochloric acid was added and refluxed at 80°C for 30 min. Neutralized solution was directly applied to HPTLC plate followed by development and scanning under optimized chromatographic conditions.

2.7.2. Base-Induced Degradation Study. To 5 mL of above methanolic drug solution, 5 mL of 0.1 M sodium hydroxide was added and refluxed at 80°C for 30 min. Neutralized solution was directly applied to HPTLC plate followed by development and scanning under optimized chromatographic conditions.

2.7.3. Hydrogen Peroxide-Induced Degradation Study. To 5 mL of methanolic drug solution, 5 mL of 3% hydrogen peroxide solution was added and refluxed at 80°C for 30 min. The resulting solution was directly applied to HPTLC plate and the chromatogram was recorded under optimized chromatographic condition.

2.7.4. Wet Degradation Study. For wet heat degradation study, the methanolic standard solution was refluxed at 80°C for 30 min. The resulting solution was directly applied to HPTLC plate followed by development and scanning under optimized chromatographic conditions.

2.7.5. Photodegradation Study. The rifaximin standard, 10 mg was exposed to UV light in a UV chamber and sunlight for 24 hours and appropriate dilutions were made in methanol to obtain final concentration of 1000 μg/mL followed by application, development, and scanning under optimized chromatographic conditions.

2.8. Degradation Kinetic Investigation. To study the acid induced degradation kinetics of rifaximin, 5 mL of the above methanolic drug solution (1 mg/mL) was refluxed at 313, 323, 333, and 343 K, after addition of 5 mL of 0.1 M hydrochloric acid. Three microlitres (750 ng) of neutralized solution was applied on HPTLC plate at various time intervals up to 90 min and chromatograms were recorded under optimized chromatographic conditions. Each experiment was repeated three times at each temperature and time interval and the concentration of the remaining drug was calculated. Further degradation kinetics was evaluated by plotting $2 + \log C_t/C_0$ versus time in minutes. Arrhenius plot [$(3 + \log K_{\text{obs}})$ value versus the reciprocal temperature $(1/T \times 10^3)$] was thenused for computing different parameters like degradation rate constant, half-life, shelf life, and activation energy. The $t_{1/2}$

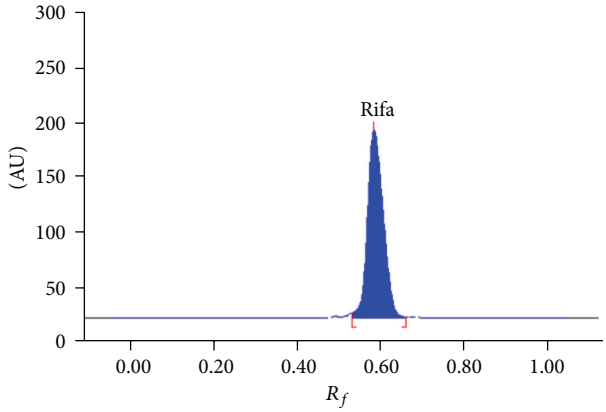

Figure 2: Chromatogram of rifaximin standard (R_f = 0.59).

and t_{90} were also calculated for acid degradation at 25°C by extrapolation from Arrhenius plot.

2.9. Statistical Analysis. Statistical parameters like standard deviation (SD) and percent relative standard deviation (% RSD) were computed by using MS Excel (Microsoft Corporation, USA). Bartlett's test was applied on calibration data for evaluation of homoscedasticity of variance.

3. Result and Discussion

3.1. Optimization of Mobile Phase. Both the pure drug and the degraded products were spotted on the HPTLC plates and run in different solvent systems and ratios, n-hexane, toluene, 2-propanol, methanol, and ethyl acetate. From these, combination of n-hexane : 2-propanol gave good results and hence further trials were initiated for different ratios of n-hexane : 2-propanol. The mobile phase n-hexane : 2-propanol (5 : 4, v/v) gave good resolution with R_f value of 0.59 for rifaximin but typical peak nature was missing. Also, the band for rifaximin was slightly diffused. Band characteristic was improved by addition of acetone (1 mL) to the above mobile phase. But considerable tailing was observed, and hence ammonia was added to minimize tailing. Finally, the mobile phase consisting of n-hexane : 2-propanol : acetone : ammonia (5 : 4 : 1 : 1, v/v/v/v) gave a sharp and symmetrical peak. Well-defined band of rifaximin at R_f 0.59 (Figure 2) was obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

Rifaximin showed good correlation over a concentration range of 400–3200 ng/band with respect to peak area (Figure 3, Table 1). The linearity of calibration curve and adherence of system to Beer's law were evaluated by high value of correlation coefficient. Further homoscedasticity of variance for response, peak area with respect to concentration range of 400–3200 ng/band was also validated by Bartlett's test. The results show that the calculated χ^2 value is less than the critical value at 95% confidence interval, $\chi^2_{(0.05,5)}$ = 9.488, thus indicating that the variance of response is homogeneous (Table 1).

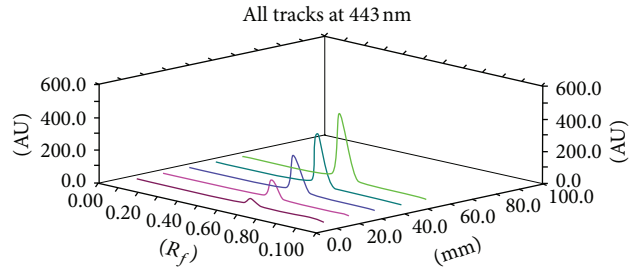

Figure 3: 3D densitogram for calibration curve linearity of rifaximin standard.

Table 1: Linear regression parameters for rifaximin by HPTLC method.

Parameters	Rifaximin
Linearity range[a] (ng/band)	400–3200
Correlation coefficient (r^2)	0.999 ± 0.0024
Slope ± SD (S_b)	4.83 ± 0.06
Confidence limit of slope[c]	4.76–4.91
Intercept ± SD (S_a)	309.82 ± 89.70
Confidence limit of intercept[c]	197.40–420.18
Limit of detection (ng/band)	61.25
Limit of quantitation (ng/band)	185.59
Bartlett's test[b] (χ^2)	0.267

[a]n = 5 replicates, [b]calculated value χ^2 less than critical value $\chi^2_{(0.05,5)}$ = 9.488, and [c]95% confidence limit.

3.2. Linearity

3.3. Analysis of Pharmaceutical Formulation. The tablets formulations, RCIFAX 400 mg, RCIFAX 200 mg, RIFAGUT 200 mg, RIXMIN 200 mg, and TORFIX 200 mg when analyzed in triplicate using the developed method, showed only one peak at R_f value of 0.59 for rifaximin in the chromatogram of tablet formulation indicating no interference of the excipient (Figure 4). The average percentage content of rifaximin found for all tablet formulation was in the range of 95–98% w/w as shown in Table 2.

3.4. Validation of Method

3.4.1. Precision. Repeatability and intermediate precision expressed in terms of % RSD reveal that the proposed method provides an acceptable intraday and interday variation as shown in Table 3.

3.4.2. Accuracy. The proposed method when used for evaluation of recovery at three concentrations levels, 50%, 100%, and 150% after spiking with standard, showed percentage recovery between 100.35% and 103.12%, with acceptable % RSD, less than 2 (Table 4).

3.4.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ). Limit of detection and limit of quantitation were

Table 2: Applicability of the proposed HPTLC method for determination of rifaximin in pharmaceutical formulation.

Drug	Brand name	Amount of rifaximin present (mg per tablet)	% amount of rifaximin found[a]	SD	% RSD
Rifaximin	RCIFAX	400	98.26	1.58	1.61
	RCIFAX	200	96.69	1.20	1.24
	RIFAGUT	200	95.59	1.68	1.76
	TORFIX	200	98.02	1.66	1.69
	RIXMIN	200	97.22	1.01	1.04

[a]Average of three replicates.

Table 3: Intra- and interday precision of HPTLC method.

Amount (ng/band)	Intraday precision[a]		Interday precision[a]	
	SD	% RSD	SD	% RSD
800	1.06	1.03	1.37	1.31
1600	0.17	0.16	0.42	0.41
3200	1.59	1.63	1.62	1.64

[a]$n = 3$ replicates.

Table 4: Recovery study for determination of rifaximin.

Recovery level (%)	Initial amount (ng/band)	Amount added (ng/band)	% recovery[a]	SD	% RSD
50	1000	500	103.12	0.12	0.12
100	1000	1000	100.35	0.28	0.28
150	1000	1500	101.10	1.39	1.38

[a]$n = 3$, average of three estimations at each level.

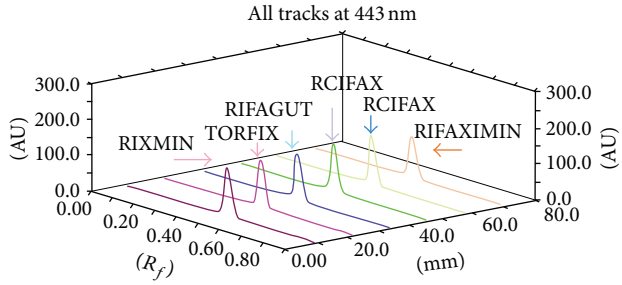

Figure 4: 3D densitogram of pharmaceutical formulations of rifaximin ($R_f = 0.59 \pm 0.03$) with rifaximin standard ($R_f = 0.59$).

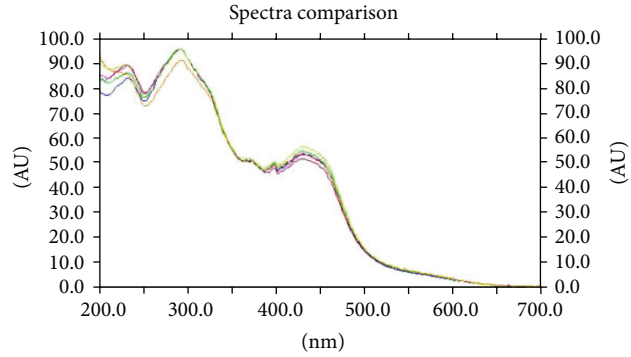

Figure 5: Overlain spectra of five pharmaceutical tablet formulations with rifaximin standard showing peak purity.

found to be 61.24 ng/band and 185.59 ng/band, indicating good sensitivity of the method.

3.4.4. Robustness. The R_f value of rifaximin was in the range of 0.59 ± 0.03, on small deliberate changes in various parameters related to mobile phase, chromatographic development, and scanning. % RSD less than 2 (Table 5) indicates the robustness of the proposed method.

3.4.5. Specificity. The chromatogram of the pharmaceutical formulation and degradation samples using the developed method showed only one peak at R_f value of 0.59 for rifaximin that was found to be at the same R_f value for rifaximin standard by comparison of chromatograms (Figure 4). The peak purity of rifaximin in tablet formulations when evaluated by comparing the overlaid spectra at peak start, peak apex, and peak end positions of the spot showed good correlation; that is, $r(S, M) = 0.9999$ and $r(M, E) = 0.9998$ (Figure 5), indicating specificity in presence of excipients. Similarly, peak purity of rifaximin in degradation study also showed good correlation; that is, $r(s, m) = 0.999$ and $r(m, e) = 0.998$, indicating method specificity in the presence of degradation product (Figure 6).

3.5. Forced Degradation Study. The densitogram of the forced degradation sample (acid, base, hydrogen peroxide, sunlight, UV light, and wet heat) showed well-separated peak of

Table 5: Robustness study of developed HPTLC method.

Parameters	SD[a]	(%) RSD
Mobile phase composition (4.9 : 3.9 : 1 : 1, 5.1 : 4.1 : 0.9 : 0.9)	1.31	1.36
Saturation time (20 ± 5 min)	0.50	0.50
Development distance (8 ± 1 cm)	1.18	1.16
Scanning time interval (1 h, 2 h, 3 h, 4 h, 5 h, and 6 h)	0.15	0.15
Wavelength change (442 ± 1 nm)	0.72	0.73
Time from spotting to chromatography (0, 20, 40, and 60 min)	0.52	0.52
Mobile phase volume (11 ± 2 mL)	1.58	1.58

[a]$n = 3$ replicates for concentration of 800 ng/band.

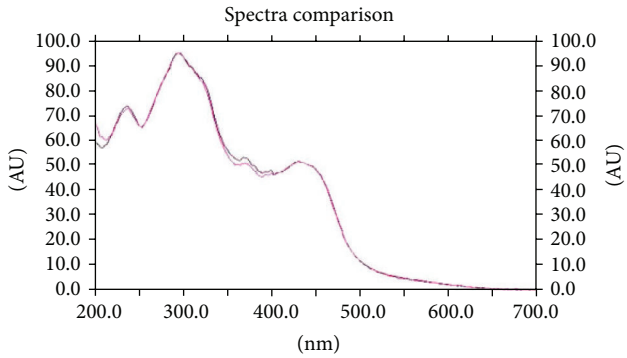

Figure 6: Overlain spectra of acid degradation sample with rifaximin standard showing peak purity.

Table 6: Degradation study of rifaximin in various conditions.

Exposure conditions	Degradation products (R_f)	Recovery (%)[a]
None (control sample)	Not detected	100
0.1 M HCl, refluxed (30 min)	0.44, 0.49	55.39
0.1 M NaOH, refluxed (30 min)	0.46, 0.51	81.94
3% Hydrogen peroxide, refluxed (30 min)	0.47	94.95
Wet heat, refluxed (30 min)	—	100
Sunlight (24 h)	0.67	89.72
UV light, 254 nm (24 h)	0.49	98.34

[a]$n = 3$ replicates for concentration of 1000 ng/band.

rifaximin standard from degradation peak at different R_f value (Table 6).

3.5.1. Acid- and Base-Induced Degradation. Recovery of rifaximin at the level of 55.39% and 81.34% from the acid and base stressed samples, respectively, suggests significant degradation of rifaximin in acid degradation compared to alkaline degradation (Table 6). The chromatogram of the acid degraded sample showed two additional peaks at R_f value of 0.44 and 0.49, respectively (Figure 7). Similarly, the

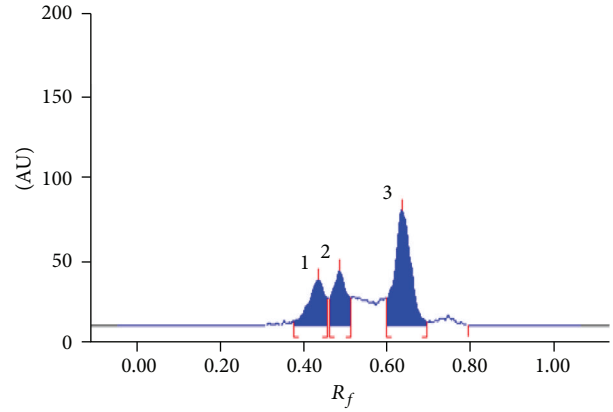

Figure 7: Chromatogram of rifaximin standard drug subjected to acid degradation (rifaximin = 0.63, $DP_1 = 0.44$, $DP_2 = 0.49$).

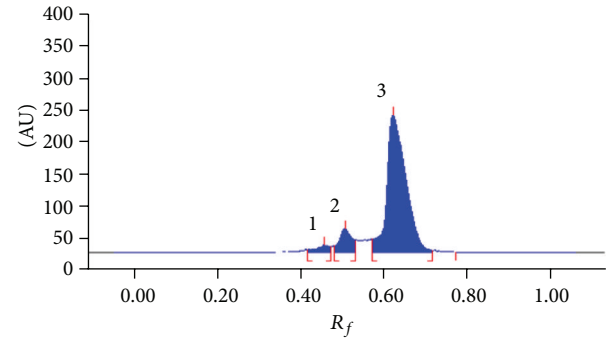

Figure 8: Chromatogram of rifaximin standard subjected to base degradation (rifaximin = 0.63, $DP_1 = 0.46$, $DP_2 = 0.51$).

chromatogram of the base-induced degradation also showed two additional peaks at R_f value of 0.46 and 0.51 (Figure 8).

3.5.2. Hydrogen Peroxide-Induced Degradation. The chromatogram of rifaximin standard when treated with 3% v/v hydrogen peroxide showed additional peak at R_f value of 0.47, suggesting that rifaximin is susceptible towards oxidation induced degradation. The area of the degradation product peak at $R_f = 0.47$ was not significant (Figure 9) and was accountable for 95.73% recovery of rifaximin (Table 6).

3.5.3. Wet Degradation Study. Rifaximin solution when heated at 80°C for 30 min showed no additional peak of degradation product suggesting stability of rifaximin in methanolic solution (Figure 10 and Table 6).

3.5.4. Photodegradation Study. Both UV and sunlight degraded samples of rifaximin showed additional peak at R_f value of 0.49 and 0.67, respectively (Figures 11 and 12). Drug recoveries at the level of 89.72 and 98.34 for the sunlight and UV exposed samples, respectively, showed that drug is unstable more towards the sunlight degradation than UV irradiation (Table 6).

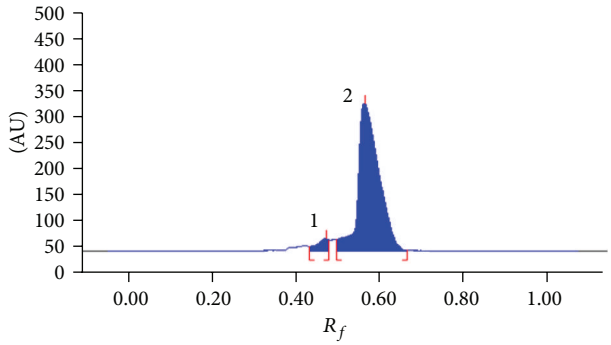

Figure 9: Chromatogram of rifaximin standard subjected to 3% hydrogen peroxide induced degradation (rifaximin = 0.56, DP = 0.47).

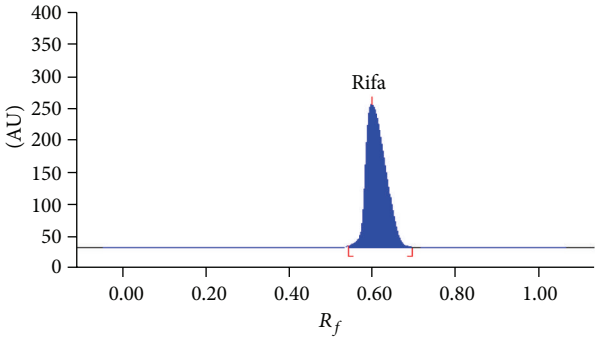

Figure 10: Chromatogram of rifaximin standard subjected to wet degradation (rifaximin = 0.60).

Table 7: Degradation rate constant (K_{obs}), half-life ($t_{1/2}$), and shelf life (t_{90}) for rifaximin at various temperatures.

Temp. (K) 0.1 M HCl	K_{obs} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)
313	0.00208	5.55	0.84
323	0.00524	2.20	0.33
333	0.01	1.05	0.15
343	0.03	0.41	0.06

3.6. Degradation Kinetic Study. At the selected temperatures of 313, 323, 333, and 343 K, the degradation process followed first-order kinetics, revealing a decrease in concentration of drug with increasing time (Figure 13).

First-order rate constant (K_{obs}), half-life ($t_{1/2}$), and shelf life (t_{90}) were also computed from the slope of the straight lines at each temperature for acidic degradation (Table 7). A plot of ($3 + \log K_{obs}$) value versus the reciprocal temperature ($1/T \times 10^3$), the Arrhenius plot (Figure 14), was found to be linear in the selected temperature range (40–70°C).

Degradation rate constant of acidic degradation process at room temperature (25 ±°C) was obtained by extrapolation from Arrhenius plot. The first-order $t_{1/2}$ and t_{90} were also calculated for acid degradation at 25°C (Table 8). Degradation kinetic study reveals that the drug is highly susceptible to acidic degradation.

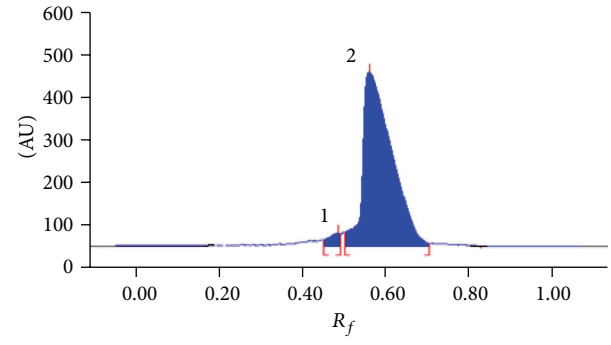

Figure 11: Chromatogram of rifaximin standard subjected to UV degradation (rifaximin = 0.57, DP = 0.49).

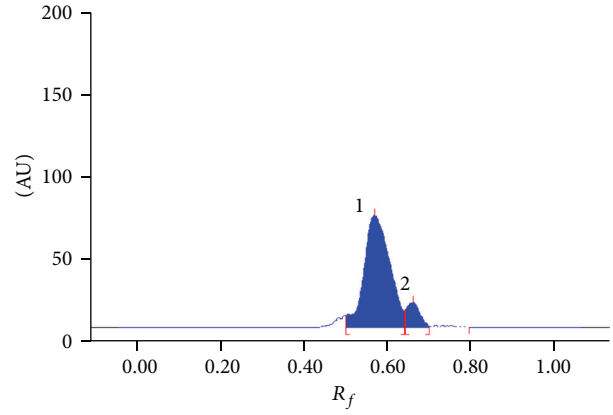

Figure 12: Chromatogram of rifaximin subjected to sunlight degradation (rifaximin = 0.57, DP_1 = 0.67).

Table 8: Degradation kinetic profile for rifaximin in acidic media at 25 ± 2°C.

Parameters	0.1 M HCl
Ea (kcal mol^{-1})	18.45
K_{25} (h^{-1})	7.4×10^{-6}
$t_{1/2}$ (h)	25.89
t_{90} (h)	3.92

4. Conclusion

A stability indicating HPTLC method was developed and validated for the determination of rifaximin. The developed method was found to be simple, specific, sensitive, and suitable for the determination of rifaximin. The proposed method was applied for the analysis of rifaximin in tablet formulation, where the results were statistically compared with the reported HPLC method [10]. The t and F values were computed and found less than the tabulated ones indicating no significant difference with respect to formulation analysis and precision, as shown in Table 9. These statistical results suggest the use of the proposed method in routine and quality control analysis without interference of commonly encountered excipients of dosage form. Moreover, cost per sample analysis is relatively low in comparison with HPLC method.

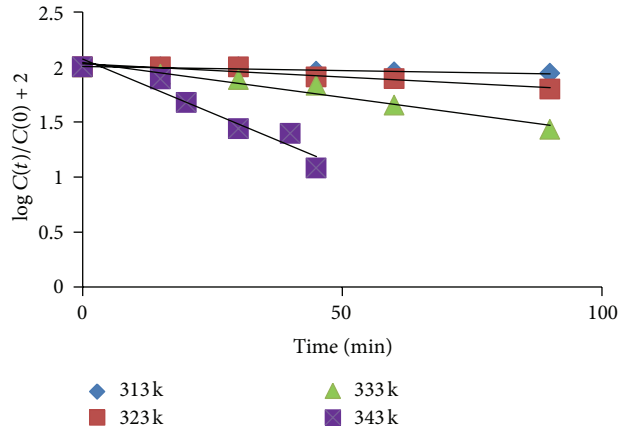

Figure 13: First-order plot for degradation of rifaximin at various temperatures: C_t concentration at time t; C_0 concentration at time zero.

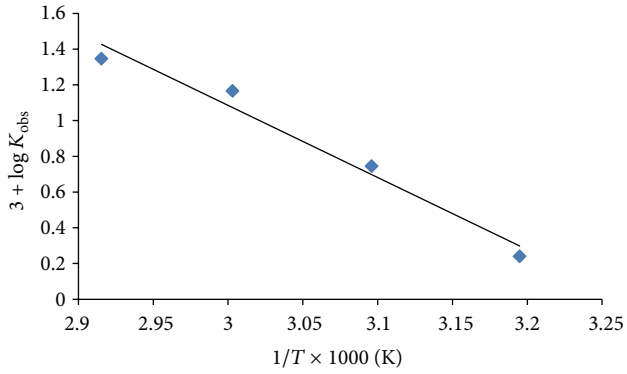

Figure 14: Arrhenius plot for the degradation of rifaximin in presence of 0.1 M HCl and their extrapolation for predicting degradation at room temperature (25 ± °C).

Table 9: Statistical comparison of the results obtained by the proposed HPTLC method in comparison to the reported HPLC method.

Parameter	HPTLC	HPLC*
Mean % found for analysis of RCIFAX tablet formulation[a]	96.69	99.59
TORFIX tablet formulation[a]		
F test (39)[b]	98.02	98.48
Formulation analysis[a]	1.15	
Intraday precision[a]	3.87	
Interday precision[a]		
t-test (2.776)[b]	3.61	
Formulation analysis[a]	2.38	
Intraday precision[a]	0.32	
Interday precision[a]	0.22	

*Reported HPLC method using sodium acetate buffer (pH 4.0): acetonitrile (35 : 65 v/v), at 441 nm; [a]average of three determinations; [b]the values in the parenthesis represent corresponding tabulated values of t and F at $P = 0.05$.

Additional advantages include high sample throughput, short system equilibration time, minimum mobile phase requirement, and no prior requirement of degassing and filtration of mobile phase. Forced degradation study showed that all degradation products were well separated from rifaximin under various conditions, thus confirming the method as stability-indicating analytical methodology and that it can be employed for the determination of rifaximin in stability studies.

Acknowledgment

The authors are highly grateful to Lupin Pharma Ltd., Mumbai, India, for providing gratis sample of rifaximin.

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