# Validated stability-indicating thin layer chromatographic determination of nadifloxacin in microemulsion and bulk drug formulations

# Validated Stability-Indicating Thin Layer Chromatographic Determination of Nadifloxacin in Microemulsion and Bulk Drug Formulations

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## ABSTRACT

A stability-indicating high performance thin layer chromatographic (HPTLC) method for the densitometric analysis of nadifloxacin in microemulsions was developed and validated according to the guidelines of the International Conference on Harmonization (ICH). The compact spot for nadifloxacin was found at an $R_f$ value of $0.39 \pm 0.02$ at an absorption wavelength of 288 nm using a mobile phase consisting of chloroform : methanol : formic acid (7.5 : 2.0 : 0.5 v/v). The linear regression data for the calibration plots ($r^2 = 0.9981$) was found with respect to peak area in the concentration range of 50 - 600 ng/spot. The limit of detection (LOD) and limit of quantification (LOQ) were 9.4 and 20.5 ng respectively. The drug was subjected to acid and alkaline hydrolysis, oxidation, photo degradation and dry heat treatment. The peaks of degradation products were well-resolved from the peak of the standard drug with significantly different $R_f$ values. Statistical analysis revealed that the developed HPTLC method is reproducible, selective and accurate for the determination of nadifloxacin in its formulations. The method can effectively separate the drug from its degradation products and be used for stability-indicating assay.

Key words: high performance thin layer chromatography, method validation, nadifloxacin, stability-indicating assay method, fluoroquinolone

## INTRODUCTION

Nadifloxacin, designated chemically as 9-fluoro-6,7-dihydro-8(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo-(ij)-quinolizine-2-carboxylic acid (OPC-7251) (Figure 1), is a second generation broad spectrum fluoroquinolone with improved activity against Gram-positive and Gram-negative bacteria, including *S. aureus, P. acnes and S. epidermidis*. The bactericidal action of nadifloxacin is mediated by inhibiting the formation of supercoiled DNA by DNA gyrase (topoisomerase II), an enzyme responsible for bacterial DNA replication[1]. It is used for the treatment of bacterial skin infection and acne vulgaris.

According to literature review, only HPLC method has been reported for the determination of nadifloxacin in plasma[2] while no analytical method has been reported for the estimation of nadifloxacin in bulk drugs and dosage forms which indicate stability. Based on the stability indication method that is reported in the International Conference on Harmonization (ICH) guideline Q1A (R2) titled "Stability testing of new drug substances and products", the stress testing of new drug substances should be carried out to elucidate the inherent stability characteristics of the active substances[3]. Oxidation, acid or base hydrolysis and photolytic degradation are some of the required tests needed to evaluate degradation
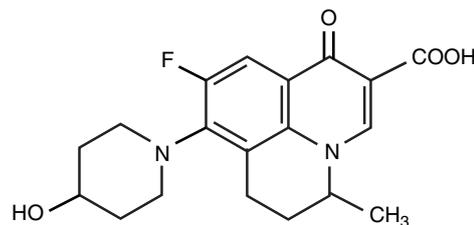


**Figure 1.** Chemical structure of nadifloxacin

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of active pharmaceutical ingredient (APIs). An ideal stability indication method should quantify the drug *per se* and also resolve its degradation products[4].

HPTLC is a powerful analytical technique because of its reliability, simplicity, reproducibility and speed. It is also an economical method that utilises relatively small amounts of solvents and minimum sample clean-up. Most importantly, a large number of samples can be quickly and simultaneously analyzed. Unlike HPLC, HPTLC has no limitation on the choice of mobile phase. Mobile phases having pH 8 and above can be employed[5]. It also allows simultaneous assay of several compounds in herbal extracts and formulations.

The main objective of the present work was to develop an economical, specific, accurate, reproducible and stability-indicating HPTLC densitometric method for the estimation of nadifloxacin, in the presence of its degradation products and related impurities, in micro-emulsion pharmaceutical dosage form and validate it according to ICH guidelines.

## MATERIALS AND METHODS

### I. *Materials*

Nadifloxacin was received as a gift sample from Wochardt Laboratory Limited (Aurangabad, India). Other chemicals and reagents used were purchased from Merck Limited (Mumbai, India) and were of analytical grade.

### II. *HPTLC Instrumentation*

The sample was spotted with a band width of 6 mm using a Camag microlitre syringe on pre-coated silica gel aluminium plates $60_{F254}$ (20 cm × 10 cm with thickness of 0.2 mm, E. Merck (Darmstadt, Germany). A sample applicator, Linomat V (Camag, Muttenz, Switzerland) was used. The slit dimension was kept at 6 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. A constant application rate of 100 nL/s was used and the band interval was 10 mm. For the application of sample by spray-on technique, the required volume with an additional 2.2 µL (internal storage volume) was filled in the syringe. The mobile phase consisted of chloroform : acetone : formic acid (7.5 : 2.0 : 0.5 v/v). Linear ascending development was carried out in a 20 cm × 20 cm twin-trough glass chamber saturated with the mobile phase for up to 30 min at room temperature (25 ± 2°C) and a relative humidity of 55 ± 5%. After the development, the TLC plates were dried in a current of air with an air dryer. Densitometric scanning was performed on Camag TLC Scanner III at an absorption wavelength of 288 nm. The source of radiation utilized was a deuterium lamp emitting continuous UV spectrum in the range of 190-400 nm. Evaluation was performed using linear regression analysis based on peak area.

### III. *Calibration of Nadifloxacin*

A stock solution of nadifloxacin (100 µg/mL) was prepared in methanol. 0.5, 1, 2, 4, 5 and 6 µL of the stock solution was applied on a TLC plate to obtain concentrations of 50, 100, 200, 400, 500 and 600 ng/spot, respectively. Each concentration was spotted three times on the TLC plate. A calibration curve was obtained by plotting peak area against the corresponding concentration and linear least-square regression analysis was performed.

### IV. *Method Validation*

Precision, robustness, limit of detection (LOD), limit of quantification (LOQ), recovery and ruggedness[6] of the developed HPTLC method was evaluated according to the ICH guideline Q2 (R1). The procedure used to study these parameters was the same as previously reported for other HPTLC methods[4,7].

#### (I) *Precision*

Precision validation was performed in two stages, namely system repeatability and method repeatability. For system repeatability, the repeatability of sample application and measurement of peak areas were carried out by analyzing six samples at two concentrations (200 and 300 ng/spot) on the same day. For method repeatability, intra and inter-day variations were studied for three different concentration levels of nadifloxacin (100, 200 and 300 ng/spot).

#### (II) *Limit of Detection (LOD) and Limit of Quantification (LOQ)*

In order to determine the detection and quantification limits, nadifloxacin drug concentrations in the lower part of calibration curve were used. Nadifloxacin solutions of 50, 100, 200 and 300 ng/µL were prepared and applied in triplicate. The amount of nadifloxacin concentration by spot versus average response (peak area) was plotted. The equation of this curve was also determined and an estimate of the target response (ybl), which corresponded to the intersection of the curve, was obtained. Subsequently, a second curve was plotted with concentration versus standard deviation of the responses. From this equation, an estimate of standard deviation of the target (sbl), which corresponded to the intersection of curve, was obtained. The limits of detection and quantification were calculated using the following formulae:

$$\text{Limit of Detection} = \frac{ybl + 3\ sbl}{b}$$

$$\text{Limit of Quantification} = \frac{ybl + 10\ sbl}{b}$$

where b = slope of the curve

(III) *Recovery Studies*

A stock solution of nadifloxacin was prepared. The microemulsion formulation was first analyzed by the proposed method. The analyzed samples were spiked with 50%, 100% and 150% of the standard drug and the mixture was re-analyzed. The experiment for each recovery sample was carried out six times to check the recovery of the drug at different formulation levels.

(IV) *Robustness*

Robustness of the developed method was determined by introducing small changes in the mobile phase composition and the effect on the result was examined. Mobile phases comsisting of different compositions of chloroform and acetone with the same formic acid concentration (7.8 : 1.7 : 0.5 v/v and 7.2 : 2.3 : 0.5 v/v ) were used. The temperature and relative humidity varied in the range of ± 5%. The plates were pre-washed with methanol and activated at 60 ± 5°C for 2, 5 and 7 min prior to use. The time from spotting to chromatographic analysis and from chromatographic analysis to scanning was varied by 0, 20, 40 and 60 min. Robustness of the method was evaluated at two different concentration levels of 200 and 400 ng/spot.

(V) *Ruggedness*

For ruggedness, a solution of 400 ng/spot of nadifloxacin was prepared and analyzed at 0, 6, 12, 24, 48 and 72 h.

V. *Analysis of Nadifloxacin in Microemulsion*

To determine the content of nadifloxacin in microemulsion, 1 mL of microemulsion (which contains 1 mg of nadifloxacin) was diluted with methanol and the final solution was diluted to obtain 100 µg/mL of nadifloxacin. Two micromilliliter (200 ng/spot) of the solution was applied on the TLC plate, followed by development and scanning as described in Materials and Methods Section II. The analysis was carried out in triplicate. The possibility of interference by excipients was observed.

VI. *Stress Degradation Study of Nadifloxacin*

For the stress degradation study of nadifloxacin, the samples were separately treated with acid, base, dry heat, day light, UV light and $H_2O_2$. The chromatograms were developed.

(I) *Acid- and Base-Induced Degradation*

Ten milligram of nadifloxacin was dissolved in 10 mL of methanol. One milliliter of the solution was used and the volume was made up to 10 mL with 0.1 N HCl

and 0.1 N NaOH separately. The solutions yielded extreme pH values of 1 and 13, respectively. These solutions were kept for 3 days at 50°C in a dark place in order to exclude the possible effect of light. The resultant solutions were applied on TLC plates in triplicate (5 µL each, i.e. 500 ng/spot).

(II) *Hydrogen Peroxide-Induced Degradation*

Ten milligram of nadifloxacin was dissolved in 10 mL of methanol. 1 mL of solution was used and the volume was made up to 10 mL with 30% v/v $H_2O_2$ and refluxed. The solutions were kept for 3 days at room temperature in a dark place in order to exclude the possible effect of light. The resultant solutions were applied on TLC plates in triplicate (5 µL each, i.e. 500 ng/spot).

(III) *Photo-Stability*

The photo-stability test was performed in the presence of UV light (254 nm) and daylight. The methanolic solution having a concentration of 100 µg/mL was kept for 3 days in UV light and for 7 days in daylight. The resultant solution was applied on TLC plates in triplicate (5 µL each, i.e. 500 ng/spot).

(IV) *Dry Heat Degradation*

The dry heat degradation study was performed using a high precision hot air oven (Narang Scientific Works, New Delhi, India) capable of controlling the temperature within ± 2°C The methanolic solution having a concentration of 100 µg/mL was kept for 8 h in an oven to expose it to dry heat (100°C). The resultant solution was applied on TLC plates in triplicate (5 µL each, i.e. 500 ng/spot).

## RESULTS AND DISCUSSION

I. *Optimization of Mobile Phase*

The TLC procedure was optimized with a view to develop a stability-indicating assay method to quantify nadifloxacin in microemulsion formulations. Chloroform was selected as one of the components of the mobile phase with acceptable resolution. However, the $R_f$ value was too low, so the solvent strength was increased by adding polar solvents. Acetone was added to chloroform in ratios of 3 : 7, 2 : 8, 1.5 : 8.5, 2.5 : 7.5 and 0.5 : 9.5 and the chromatograms were developed. The mobile phase comprising of chloroform : acetone (7.5 : 2.5 v/v) showed good resolution with $R_f = 0.39$ for nadifloxacin but tailing was observed and the spot of nadifloxacin was slightly diffused. Addition of formic acid improved the characteristics of the spot and its optimum quantity was 0.5 mL. The final mobile phase selected was a mixture of chloroform : acetone : formic acid (7.5 : 2 : 0.5 v/v), which

gave a well-defined symmetrical peak of nadifloxacin at $R_f = 0.39 \pm 0.02$ (Figure 2). Well-defined spots were obtained when the chamber was saturated with mobile phase for 0.5 h at room temperature.
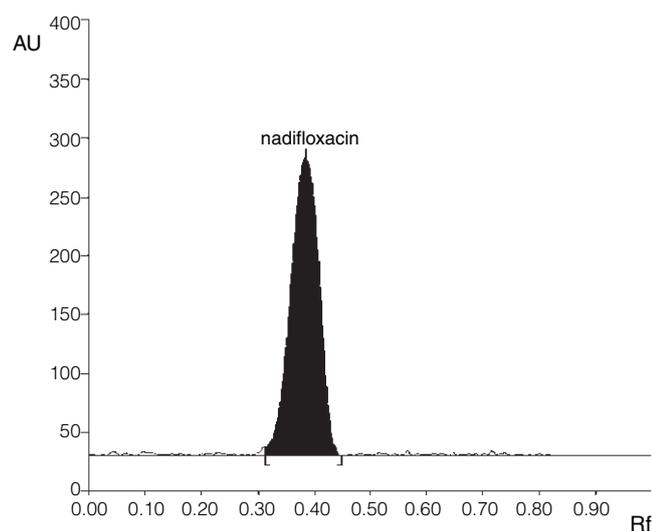
## II. *Calibration Curve*

The linear regression data for the calibration curves (n = 3) shown in Table 1 indicated a good linear relationship in the concentration range of 50 - 600 ng/spot with respect to the peak area and height. No significant difference was observed in the slopes of the standard curves (ANOVA, $p < 0.05$).

## III. *Method Validation*

### (I) *Precision*

The repeatability of sample application and peak areas measured were expressed in terms of % RSD and the results revealed good system repeatability, intra and inter-day precision (Table 2 and 3). The % RSD for system repeatability of sample application (200 and 300 ng/spot) was measured and found to be 0.12 and 0.10,

respectively. This indicated that the system performance was very good and suitable for nadifloxacin analysis. The measurement of peak area at three different concentration levels (100, 200 and 300 ng/spot) showed low values of S.E. and % RSD for inter and intra-day variations, suggesting that the method had excellent precision.

### (II) *Limit of Detection (LOD) and Limit of Quantification (LOQ)*

The calibration curve of analyte versus average response (peak area) was plotted. The regression equation was obtained (y = 15.021x + 68.78) with a regression coefficient of 0.9972. The limit of detection and limit of quantification were determined by the method described in Materials and Methods Section IV-II. From the graphs obtained between concentration and average response, and between concentration and standard deviation, the ybl and sbl values were found to be 68.78 and 23.91, respectively. Therefore, the limit of detection was found to be 9.4 ng and the limit of quantification was found to

**Table 1.** Linear regression data for the calibration curves (n = 3)

| Parameters | With Respect to the Mean Area |
|---|---|
| Linearity range | 50 - 600 |
| Correlation coefficient | 0.9981 |
| Slope ± S.D. | 16.293 ± 0.08 |
| Confidence limit of slope[a] | 16.05 - 16.45 |
| Intercept[b] ± S.D. | 134.77 ± 1.39 |
| Confidence limit of Intercept[a] | 131.42 - 137.86 |

[a] 95% confidence limit
[b] Percentage bias of intercept = -0.013



**Figure 2.** A typical HPTLC chromatogram of standard nadifloxacin (400 ng/spot, $R_f = 0.39$). The mobile phase consisted of chloroform : methanol : formic acid (7.5 : 2.0 : 0.5 v/v).

**Table 2.** System repeatability data for precision study of the HPTLC method (n = 6)

| Amount (ng/spot) | Mean Area | SD | RSD (%) | Standard Error |
|---|---|---|---|---|
| 200 | 3272.35 | 3.61 | 0.12 | 2.08 |
| 300 | 4491.00 | 4.51 | 0.10 | 2.60 |

**Table 3.** Intra and inter-day precision data of the HPTLC method (n = 6)

| Amount (ng/spot) | Intra-day precision | | | | Inter-day precision | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean Area | S.D. | RSD (%) | S.E. | Mean Area | S.D. | RSD (%) | S.E. |
| 100 | 1194.47 | 2.83 | 0.24 | 1.64 | 1322.87 | 3.18 | 0.24 | 1.84 |
| 200 | 3436.72 | 3.06 | 0.09 | 1.83 | 3297.81 | 3.29 | 0.10 | 1.90 |
| 300 | 4535.46 | 2.84 | 0.06 | 1.64 | 4528.00 | 4.58 | 0.10 | 2.65 |

be 20.5 ng, indicating that the method was adequately sensitive.

### (III) *Recovery Studies*

The proposed method was used for extraction and subsequent estimation of nadifloxacin from the micro-emulsion formulation after spiking known amounts of the drug (50, 100 and 150%) into the formulation. Accuracy was determined based on the amount of drug recovered. The results were shown in Table 4 and it was concluded that the present method was accurate for the estimation of nadifloxacin in pharmaceutical dosage forms, especially microemulsions.

### (IV) *Robustness*

The low values of RSD and S.E. obtained after introducing a small change in mobile phase composition indicated robustness of the method (Table 5). There was no significant variation in the slope values (ANOVA, $p < 0.05$).

### (V) *Ruggedness*

A low RSD value of 0.09% from the peak areas obtained by analyzing the same drug solution at a concentration of 400 ng/spot after 0, 6, 12, 24, 48 and 72 h showed ruggedness of the method.

### IV. *Analysis of Nadifloxacin in Microemulsion Formulation*

When the formulation was analysed, three spots at $R_f$ 0.18, 0.39 and 0.64 (Figure 3) were observed in the chromatogram. The other spots might belong to the excipients present in the formulation. The drug content was found to be 99.5% with RSD and S.E. of 0.15% and
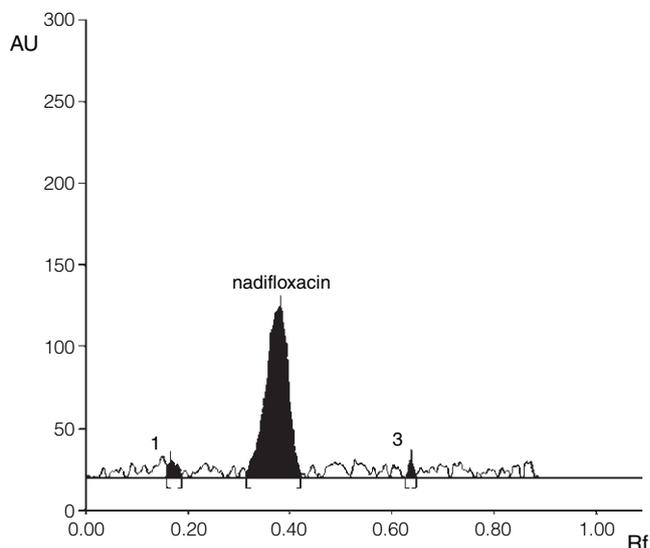


**Figure 3.** Chromatogram of nadifloxacin and the excipients in microemulsion formulation

**Table 4.** Recovery studies[a] (n = 6)

| Excess Drug Added to Analyte (%) | Theoretical Amount (ng) | Amount Recovered (ng) | Recovery (%) | RSD (%) | S.E. |
|---|---|---|---|---|---|
| 0 | 100 | 99.2 | 99.2 | 0.80 | 1.36 |
| 50 | 150 | 148.6 | 99.1 | 0.89 | 1.63 |
| 100 | 200 | 199.0 | 99.5 | 0.70 | 1.71 |
| 150 | 250 | 249.8 | 99.9 | 0.31 | 1.73 |

**Table 5.** Robustness of the method (n = 3)

| Parameters | S.D. of Peak Area | RSD (%) | S.E. | $R_f$ Value ± S.D. |
|---|---|---|---|---|
| Mobile phase composition | 1.34 | 0.12 | 0.37 | 0.39 ± 0.07 |
| Volume of mobile phase | 1.13 | 0.10 | 0.41 | 0.39 ± 0.06 |
| Temperature | 1.11 | 0.08 | 0.29 | 0.39 ± 0.05 |
| Relative humidity | 0.94 | 0.08 | 0.27 | 0.39 ± 0.06 |
| Plate pre-treatment | 0.89 | 0.07 | 0.25 | 0.39 ± 0.06 |
| Time from spotting to chromatographic analysis | 0.59 | 0.05 | 0.17 | 0.39 ± 0.04 |
| Time from chromatographic analysis to scanning | 0.47 | 0.03 | 0.16 | 0.39 ± 0.05 |

**Table 6.** Stress degradation study of nadifloxacin (n = 3)

| No. | Sample Exposure Condition | Time (Days) | $R_f$ Value of Degradation Products | Nadifloxacin Remaining ± S.D. (n = 3, 500 ng/spot) | S.E. | Recovery (%) |
|---|---|---|---|---|---|---|
| 1 | Acid, 0.1N HCl (50°C) | 3 | 0.64, 0.68, 0.71 | 161.02 ± 1.74 | 1.26 | 32.6 |
| 2 | Base, 0.1N NaOH (50°C) | 3 | 0.19, 0.42, 0.47, 0.50 | 388.44 ± 1.89 | 1.32 | 77.7 |
| 3 | $H_2O_2$, 30% v/v (25°C) | 3 | 0.06, 0.43, 0.51, 0.64 | 407.50 ± 3.2 | 1.57 | 81.5 |
| 4 | Dry heat (100°C) | 8 h | 0.42, 0.48, 0.53 | 356.33 ± 2.83 | 1.44 | 71.6 |
| 5 | Photostability - Daylight | 7 | 0.06, 0.54, 0.58 | 372.61 ± 4.12 | 1.64 | 74.5 |
| 6 | Photostability - UV light (254 nm) | 3 | 0.03, 0.27, 0.49, 0.63 | 295.53 ± 2.83 | 1.38 | 59.1 |
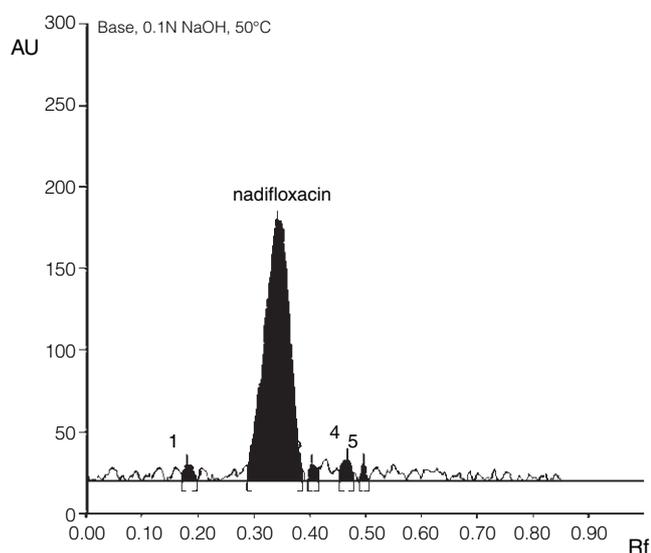


**Figure 4.** HPTLC chromatogram of base-degraded products of nadifloxacin



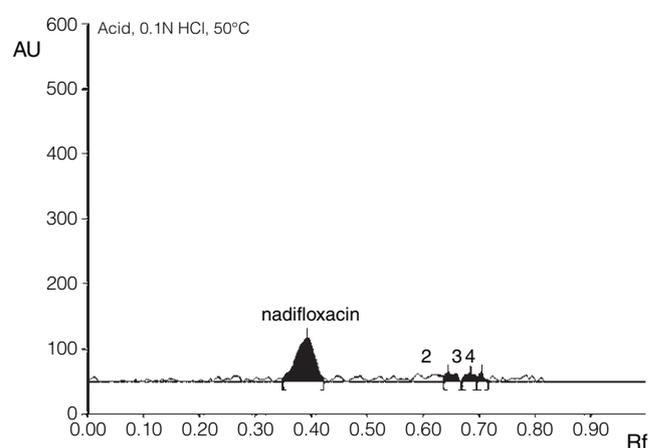**Figure 5.** HPTLC chromatogram of acid-degraded products of nadifloxacin

1.79, respectively. The results suggested that degradation of nadifloxacin had not occurred in the formulation when analyzed by this method and excipients did not affect the determination of the drug in the formulation. The low RSD value supported the suitability of this method for routine analysis of nadifloxacin in the novel drug delivery system.

### V. *Stress Degradation Study of Nadifloxacin*

The chromatogram of the samples degraded by acid, base, hydrogen peroxide, dry heat, UV light and day light showed well-separated spots of nadifloxacin, as well as some additional peaks at different $R_f$ values (Table 6).

### (I) *Acid- and Base-Induced Degradation*

It was found that the acid and base-treated samples showed different degradation products at different $R_f$ values. The base degradation products were found at $R_f$ 0.19, 0.42, 0.47 and 0.50 (Figure 4). The area of the base degradation product at $R_f$ 0.47 was found to be more significant, compared to the other three degradation products. The recovery of nadifloxacin in the base-treated sample was found to be 77.7%.

The acid-treated drug sample showed three degradation products at $R_f$ 0.64, 0.68 and 0.71 (Figure 5). The spots of degradation products were well-resolved from the drug spot. The recovery of nadifloxacin in the acid-treated sample was found to be 32.6%. From the recovery study, more degradation of nadifloxacin was observed in the acidic medium, compared to the basic medium.

### (II) *Hydrogen Peroxide-Induced Degradation*

The chromatogram of the sample treated with 30% v/v $H_2O_2$ showed four additional peaks at $R_f$ 0.06, 0.43, 0.51 and 0.64 (Figure 6). The recovery of nadifloxacin in the $H_2O_2$-treated sample was found to be 81.5%.

### (III) *Photo-Stability*

When the chromatogram was developed and evaluated, it showed a well-separated spot of pure nadifloxacin as well as some additional peaks of different $R_f$ values.

The additional peaks of different $R_f$ values 0.03, 0.27, 0.49 and 0.63 (Figure 7) and 0.06, 0.54, 0.58 (Figure 8) might belong to degraded products of nadifloxacin which were well-resolved from the pure drug in UV light and day light respectively. The recovery of drug was found to be 59.1% in UV light and 74.5% in day light. From the recovery study, the drug showed significant degradation in UV light and day light.

### (IV) *Dry Heat Degradation*

The chromatogram of the dry heat-treated sample showed three additional peaks at $R_f$ values of 0.43, 0.49 and 0.53 (Figure 9) which might belong to the degraded products of the drug. The recovery of the drug was found to be 71.3%.

From the stress degradation study of nadifloxacin, it was observed that the drug showed maximum degradation in acidic medium and UV light.

### CONCLUSIONS

The introduction of HPTLC estimation in pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPTLC method is precise, accurate, specific and stable. Statistical analysis indicated that the method is repeatable and selective for
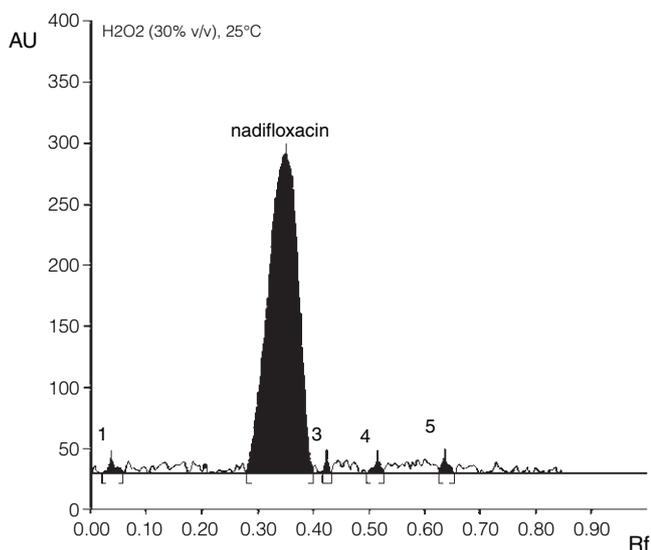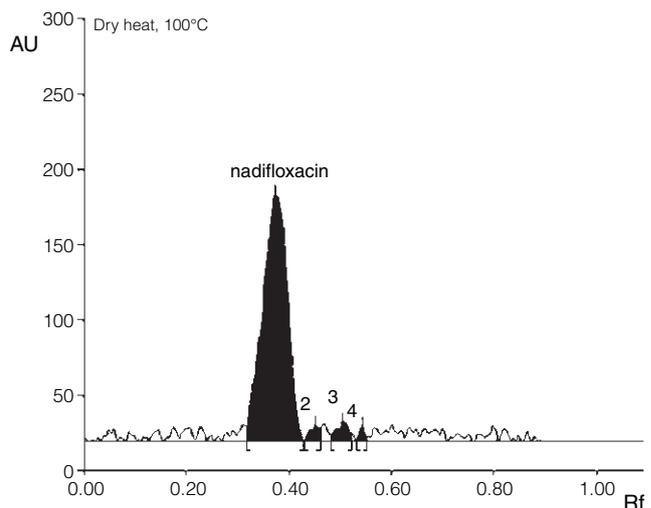


**Figure 6.** HPTLC chromatogram of $H_2O_2$-degraded products of nadifloxacin
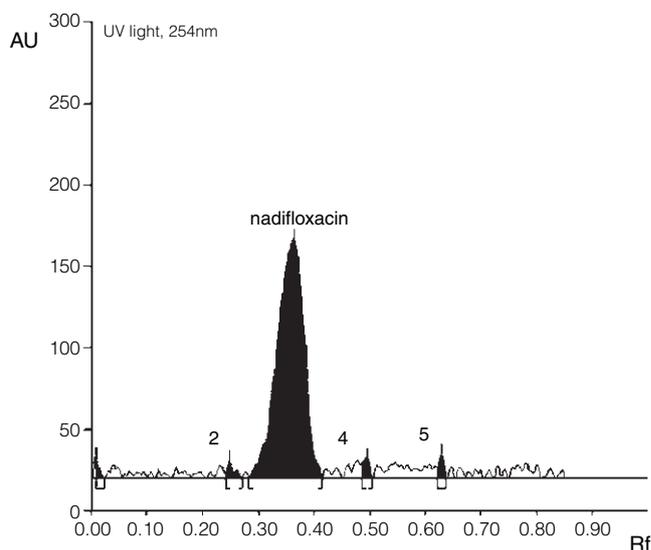


**Figure 7.** HPTLC chromatogram of UV light-degraded products of nadifloxacin
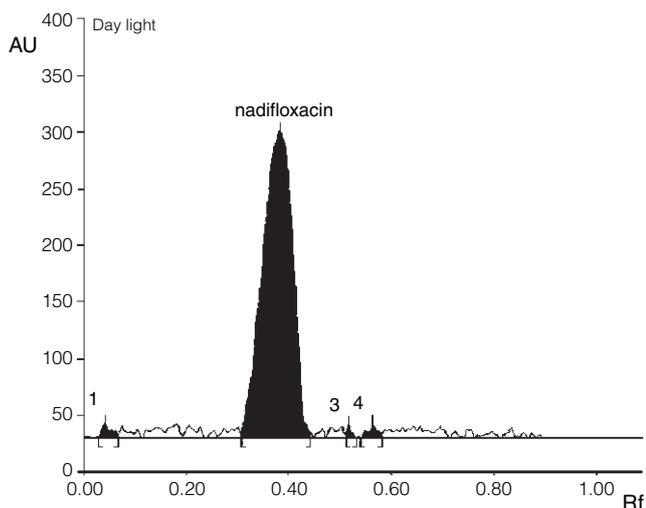


**Figure 8.** HPTLC chromatogram of day light-degraded products of nadifloxacin



**Figure 9.** HPTLC chromatogram of dry heat-degraded products of nadifloxacin

the analysis of nadifloxacin in pharmaceutical formulations with no interference from excipients. The method can be used to determine the purity of drug available from various sources by analyzing it for related substances and impurities. This method is proposed for the analysis of nadifloxacin and its degradation products in stability samples in industries. It may be further extended for quantitative estimation of nadifloxacin in plasma and other biological fluids. As the method is capable of separating the drug from its degradation products, it can be employed as a stability-indicating assay method.

## ACKNOWLEDGMENTS

## REFERENCES

 1. Nenof, P. 2006. Acne vulgaris and bacterial skin infections: review of the topical quinolone nadifloxacin. Expert Rev. Dermatol. 12: 643-654.
 2. Koike, M., Akiyama, H. and Shimizu, T. 1990. High-performance liquid chromatographic procedure for the determination of rat plasma concentrations of a new antibacterial agent, (+-)-9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid, for topical use. J. Chromatogr. 526: 235-239.
 3. ICH-Q1A (R2) Guideline. 2003. Stability testing guidelines: Stability testing of new drug substances and products. International Conference on Harmonization.
 4. Motwani, S., Khar, R. K., Ahmad, F. J., Chopra, S., Kohli, K. and Talegaonkar, S. 2007. Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin. Anal. Chim. Acta. 582: 75-82.
 5. Chopra, S., Ahmad, F. J., Khar, R. K., Motwani, S. K., Mahdi, S., Iqbal, Z. and Talegaonkar, S. 2006. Validated high-performance thin-layer chromatography method for determination of trigonelline in herbal extract and pharmaceutical dosage form. Anal. Chim. Acta. 577: 46-51.
 6. ICH-Q2 (R1). 2005. Validation of analytical procedure: test and methodology, International Conference on Harmonization.
 7. Ansari, M. J., Ahmad, S., Kohli, K., Ali, J. and Khar, R.K. 2005. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. J. Pharm. Biomed. Anal. 39: 132-138.