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STABILITY-INDICATING HPTLC METHOD FOR ESTIMATION OF LOMEFLOXACIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: The present work describes a stability-indicating HPTLC method for analysis of Lomefloxacin Hydrochloride in bulk and pharmaceutical dosage form. Precoated silica gel 60 F_{254} plate was used as stationary phase. The separation was carried out using Chloroform: Methanol: Ammonia (10: 7: 3 v/v/v) as mobile phase. The densitometric scanning was carried out at 288 nm. The linearity was obtained in the range 50–250 ng /band with correlation coefficients ($r^2 = 0.9958$). The method was validated as per ICH guidelines. Lomefloxacin Hydrochloride was subjected to forced degradation by acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drug with significantly different RF values.
KEY WORDS: Lomefloxacin Hydrochloride; HPTLC; Validation; Stability Studies.

INTRODUCTION

Lomefloxacin Hydrochloride (LOM), chemically know as (1-ethyl-6, 8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid), is a Fluoroquinolone antibiotic used for the treatment of bacterial infections of the respiratory tract, urinary tract and as a pre-operative prophylactic to prevent urinary tract infection [1 - 2]. Several types of analytical methods have been reported for the analysis of Lomefloxacin Hydrochloride in plasma and pharmaceuticals formulations, like UV-spectroscopy [3-5], HPLC [6 - 11], polarography [12], voltammetry [13], capillary electrophoresis [14], spectrofluorometry [15]. But no HPTLC method is reported so far for the estimation of drug in pharmaceutical formulations, hence we have developed a stability indicating HPTLC method for the estimation of lomefloxacin in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

MATERIALS

Lomefloxacin Hydrochloride was supplied as a gift sample by Wintac Ltd. All chemicals and reagents used were of HPLC/AR grade.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

The standard solution ranging from 50-250 ng/band was applied on precoated silica gel 60 F_{254} plate in the form of bands with 100 µl sample syringe using automatic sample applicator LINOMAT V. It was developed in a twin trough glass chamber which was already saturated for 30 min. with the mobile phase. The mobile phase consisted of Chloroform: Methanol: Ammonia (10: 7: 3 v/v/v). After development, plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved band of drug was scanned at 288 nm with Camag TLC scanner III densitometer controlled by WINCAT's software version 4.

STANDARD SOLUTIONS AND CALIBRATION GRAPHS

Stock solution was prepared by dissolving 100 mg of LOM in 100 ml distilled water, from which 1 ml was further diluted to 100 ml with methanol to get stock solution of 10ng/µl. The standard solutions were applied to reach a concentration range 50–250 ng/band for LOM. The plate was developed on previously described mobile phase and well resolved band of drug was scanned at 288 nm with scanner. The peak areas were plotted against the

corresponding concentrations to obtain the calibration curve.

ANALYSIS OF MARKETED FORMULATION:

Twenty tablets were weighed, finely powdered and powder equivalent to 100 mg LOM was transferred into 100 ml volumetric flask, to this 30 ml of distilled water was added and sonicated for 30 min. The volume was then made up to the mark using same solvent. The solution was filtered through Whatman paper No. 41. From the filtrate 1 ml was further diluted to 100 ml with methanol to get sample stock solution of LOM 10 ng/µl. Sample solution were applied six times on TLC plate to give spot concentration 100 ng/band of LOM. The plate was developed in the previously described chromatographic conditions. The peak area of the spots was measured at 288 nm and concentrations in the samples were determined using multilevel calibration.

METHOD VALIDATION

The method was validated in compliance with ICH guidelines.

SPECIFICITY

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Lomefloxacin Hydrochloride in sample was confirmed by comparing the *R*f and spectra of the spot with that of standard. The peak purity of LOM was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

PRECISION

The precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration. The intra and inter-day precision was determined by assay of the sample solution on the same day at different time intervals and on different days respectively

RECOVERY STUDIES

A recovery study was carried out by standard addition method. LOM corresponding to 80, 100 and 120% of label claim had been added to the preanalysed tablet sample solution. At each level of recovery three determinations were performed.

ROBUSTNESS

To study the robustness of the method, small but deliberate variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 15, 20 min), time from development to scanning (0, 30, 60, 90 min) were carried out.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts and slope of the regression lines were used.

FORCED DEGRADATION STUDIES:

In order to ensure that the analytical method was stability indicating, stress studies were performed.

ACID DEGRADATION STUDIES: 1 ml of 0.1N hydrochloric acid was added to 99 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

ALKALI DEGRADATION STUDIES: 1 ml of 0.1N sodium hydroxide was added to 99 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

OXIDATION STUDIES: 1 ml of a 3% hydrogen peroxide solution was added to 99 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

TEMPERATURE STRESS STUDIES: A drug solution containing 10µg/ml of drug was maintained at 50°C for 24 hrs.

RESULT AND DISCUSSION

OPTIMIZATION OF PROCEDURES

Different proportions of methanol, chloroform, Ammonia were tried while mobile phase selection. Ultimately Chloroform: Methanol: Ammonia (10: 7: 3 v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of LOM was obtained as shown in fig 1. Peak was symmetrical in nature and no tailing was observed when plates were scanned at 288 nm.

LINEARITY

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 50–250 ng /band. (r^2 = 0.9958). The standard calibration curve is given in fig 2 and standard calibration data for LOM is given in table No. 1 & 2.

ANALYSIS OF THE MARKETED FORMULATION

The spot at Rf 0.74 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The Lomefloxacin Hydrochloride content was found to be close to 100% and the results are summarized in table3. The low %RSD value indicated the suitability of this method for routine analysis.

SPECIFICITY

Good correlation was obtained between standard and sample spectra of Lomefloxacin Hydrochloride. The comparative spectrum of standard and sample is given in fig.3.

PRECISION

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 4.

RECOVERY STUDIES

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard LOM was added to pre-analyzed samples and

were subjected to the proposed HPTLC method. Results of recovery studies are shown in table 5.

ROBUSTNESS

The robustness of the method with determined by variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 15, 20 min), time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed at a concentration level of 10 µg/band of CPT, to study the effect on the peak area of the drugs. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that method is robust.

STABILITY-INDICATING PROPERTY

HPTLC studies of the samples obtained during the stress testing of LOM under different conditions using Chloroform: Methanol: Ammonia (10: 7: 3 v/v/v) as the mobile phase suggested the following degradation behavior. The amount of drug recovered after degradation studies and the Rf of degradation products are given in Table 7.

ACID-INDUCED DEGRADATION

The drug was degraded in acidic condition and shows degradation product at Rf 0.77 as shown in Figure 4.

BASE-INDUCED DEGRADATION

The drug was degraded in alkaline condition and shows degradation product at Rf 0.81 as shown in Figure 5.

HYDROGEN PEROXIDE-INDUCED DEGRADATION

The drug was degraded in hydrogen peroxide (3%) at room temperature and shows degradation product at Rf 0.79 as shown in Figure 6.

HEAT DEGRADATION

The drug when subjected to heat was degraded and degradation product appeared at Rf 0.84 and 0.91 as shown in Figure 7.

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of the stress studies indicated the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise and selective and can be employed successfully for the estimation of Lomefloxacin Hydrochloride in tablet formulation.

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Table 1: Standard calibration data for LOM (n = 3)

Concentration (ng/band)	Mean Area ± SD
50	4130.57 ± 37.32
100	5771.57 ± 42.36
150	7068.39 ± 30.82
200	8207.78 ± 90.50
250	9527.69 ± 65.98

Table 2: Linear regression data for calibration curves

Detection Wavelength (nm)	288
Beer's Law Limit (ng/band)	50-250
Regression equation	y = 26.461x + 2972.1
Correlation Coefficient (r)	0.9958
Intercept (c) ± SD	2972.1 ± 76.40
Slope (m) ± SD	26.461 ± 0.65

Table 3: Results of marketed formulation analysis

Marketed formulation	Label claim (mg)	Area* of densitogram	Amt. of drug estimated (mg) ± S.D*	% Mean amount estimated*± S.D*
Lomoflox (Ipca Labs.)	400	5646.6	404.30 ± 5.91	101.07 ± 1.48

*Average of six determinations

Table 4: Statistical evaluation of precision of developed method (n = 3)

Drug - LOM	Repeatability*	Precision	
		Intraday*	Interday*
Conc.(ng/band)	100	100	100
Mean area ± SD	5646.60 ± 39.07	5645.92 ± 37.02	5620.03 ± 0.36
% Content ± SD	101.07± 1.48	101.05± 1.40	100.07 ± 0.76
RSD (%)	1.46	1.38	0.75
S.E.	0.6034	0.5712	0.3084

*Average of six determinations

Table 5: Result from recovery studies (n = 3)

Level of recovery (%)	Amount taken (ng/band)	Amt of std added (ng/band)	Total amt recovered (ng/band)	% Recovery*	SD	S.E.	% COV
80	100	80	182.52	102.52	0.94	0.5401	0.91
100	100	100	202.12	102.12	1.10	0.6349	1.08
120	100	120	222.91	102.91	1.43	0.8238	1.39

*Average of three determinations

Table No.6: Results of robustness studies

A: Chromatographic Changes (% of chloroform in mobile phase)

% change in mobile phase	Rf	Peak area
+2%	0.71	5668.90
0%	0.73	5659.70
-2%	0.74	5593.80
Mean*± S.D.	0.726 ± 0.015	5640.80 ± 40.96

***Average of three determinations**

B: Chromatographic Changes (chamber saturation)

Chamber saturation (Time in min.)	Rf	Peak area
33	0.75	5636.00
30	0.74	5675.27
27	0.73	5659.73
Mean*± S.D.	0.74 ± 0.01	5659.73 ± 32.39

***Average of three determinations**

C: Chromatographic Changes (development distance)

development distance (mm)	Rf	Peak area
88	0.78	5662.20
80	0.77	5640.43
72	0.74	5670.73
Mean*± S.D.	0.76 ± 0.02	5657.79 ± 15.62

***Average of three determinations**

D: Chromatographic Changes (Time from application to development)

Time from application to development	Rf	Peak area
0	0.74	5661.37
10min	0.74	5656.37
20min	0.75	5643.93
30min	0.75	5621.43
Mean*± S.D.	0.75 ± 0.01	5645.78 ± 17.81

***Average of three determinations**

E: Chromatographic Changes (Time from development to scanning)

Time from development to scanning	Rf	Peak area
0	0.74	5662.0
10min	0.74	5662.0
20min	0.74	5662.0
30min	0.74	5662.0
Mean*± S.D.	0.74 ± 0	5662.0 ± 0

***Average of three determinations**

Table No. 7: System suitability parameters

Parameter	LOM
Retention time(min.)	0.74
Limit of detection (ng)	9.53
Limit of quantitation (ng)	28.87

Table 8: Results of forced degradation studies

Stress condition	Time (hours)	% Assay of active substance	Mass balance (% assay + % degradation products)	R_f values of degradation products
Acid hydrolysis (0.1 M HCl)	24	72.21	101.12	0.81
Base hydrolysis (0.1 NaOH)	24	66.21	100.99	0.77
Oxidation (3% H_2O_2)	24	46.98	100.89	0.79
Thermal degradation (50^{0}C)	24	23.43	101.01	0.84, 0.91

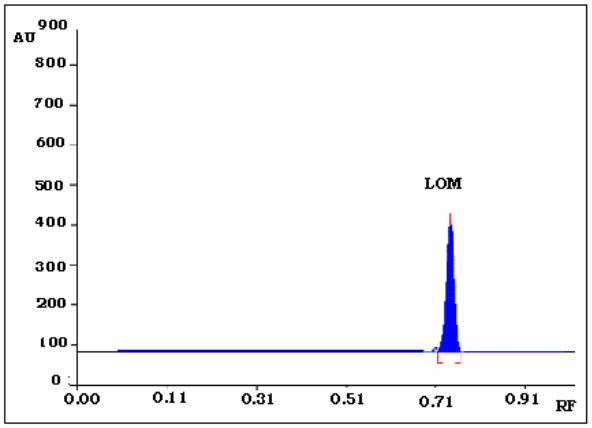

Figure 1: Densitogram of Lomefloxacin Hydrochloride

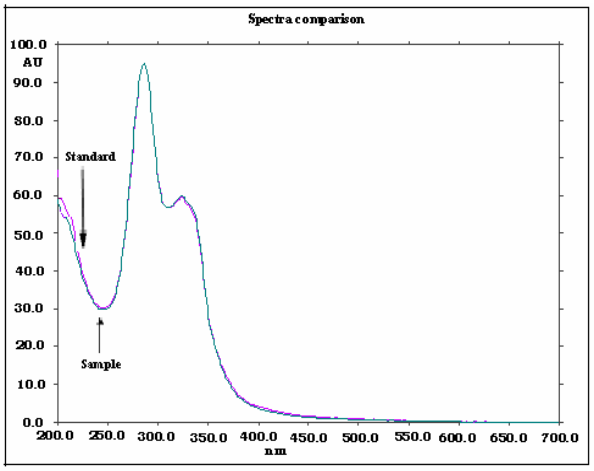

Figure 3: Spectrum of LOM standard and sample measured from 200 to 400 nm.

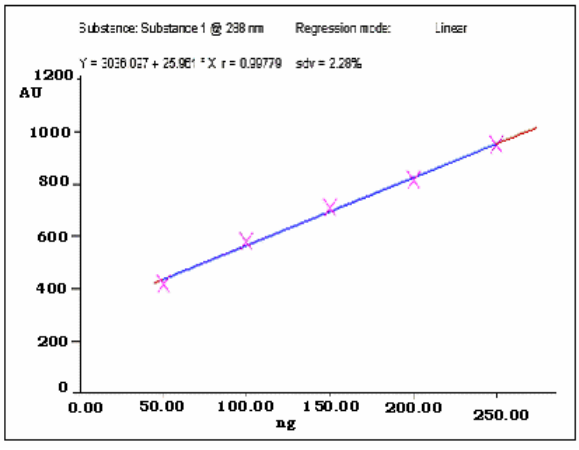

Figure 2: Calibration-curve of Lomefloxacin Hydrochloride

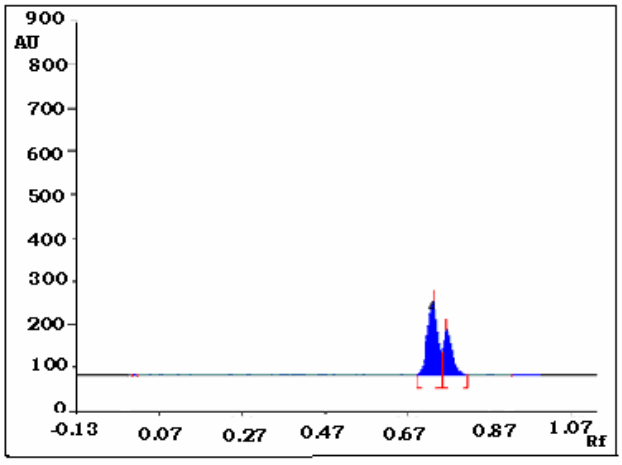

Figure 4: Acid degradation

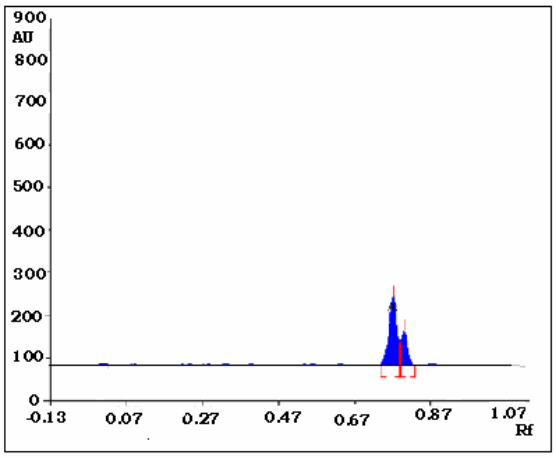

Figure 5: Alkali degradation

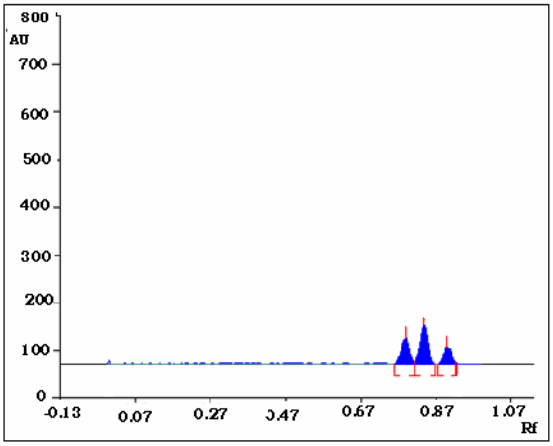

Figure 7: Heat degradation

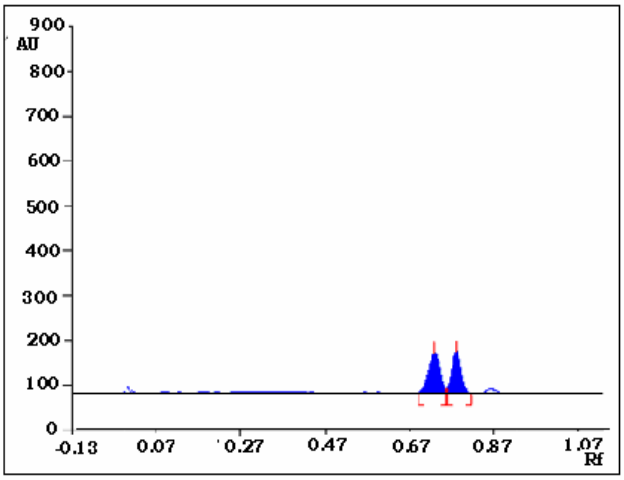

Figure 6: H_2O_2 degradation

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