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Estimation of *Lomefloxacin hydrochloride* in Bulk and Tablet dosage form by HPTLC method

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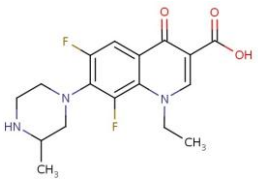
Abstract

Plan: *An analytical method for the estimation of Lomefloxacin in bulk drug and pharmaceutical dosage forms by HPTLC is described.*
Prologue: *The method is simple, fast and accurate and can be used for routine analysis of commercial Lomefloxacin tablets.*
Methodology: *The developed method used precoated silica gel 60F_{254} as stationary phase. The mobile phase used was a mixture of 2-Propanol: water: ammonia (86:8:6 %v/v/v). The RF value was 0.14.The detection of spots was carried out at 288nm. The calibration curve was found to be linear between 10 and 70 ng/spot with a correlation coefficient of 0.9969. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 5 ng/spot and 10 ng/spot respectively.*
Outcome: *The proposed method has been applied successfully for the determination of Lomefloxacin in pharmaceutical dosage forms. No significant interference was observed from excipients, coloring and flavoring agents commonly used in the formulation. The mean recovery of drug from tablets was in the range of 100.8% to 102.7 %.*
Key Words: *HPTLC, Lomefloxacin, Mobile phase, Stationary phase*.

1. Introduction

Lomefloxacin is an antibacterial drug with wide antibacterial spectrum[1]. Chemically Lomefloxacin hydrochloride is 1-Ethyl-6,8 difluoro-1-4 dehydro-7-(3-methyl-1-piperazinyl)-4-oxo-3 quinoline carboxylic acid mono Hydrochloride(Figure.1). The diflourination at positions 6 and 8 of the quinolone ring and a piperazinyl ring at 7 carrying a methyl group improve the activity spectrum and also pharmacokinetics[2].Complementing this broad antibacterial activity are excellent pharmacokinetics of Lomefloxacin including almost complete absorption, good tissue distribution, prolonged half life and significant post antibiotic effect permitting once daily administration.



Lomefloxacin hydrochloride

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Lomefloxacin is active against enterobacteriaceae and other Gram negative bacilli at MIC of 1.0 mcg/ml.Like all quinolones, lomefloxacin exerts its antimicrobial action by inhibiting the bacterial enzyme DNA Gyrase. The literature survey revealed that a few HPLC, LC-MS, GC-MS and HPTLC methods have been reported for the estimation of Lomefloxacin Hydrochloride .The methods reported are sophisticated, but costly and time consuming. The HPTLC method has the advantage that the substance placed on the plate will remain there avoiding detection problems associated with non elution and demasking by solvent front. The applications in HPTLC have been greatly speeded up by the use of automated sample application device and accurate densitometers.

2. Materials and Methods

2.1. Chemicals and Reagents

Lomefloxacin Hydrochloride working standard was procured as gift sample from Dr.Reddy's Laboratories Ltd, Hyderabad.Silica gel 60F 254 TLC plates (E.Merck, Mumbai) were used as a stationary phase. Tablets containing 400 mg each of Lomefloxacin Hydrochloride was purchased from the local market (Mahaquin400mg and Lomaday 400mg)). A Camag HPTLC system comprising of Camag Linnomat-IV automatic sample applicator, Hamilton syringe, Camag TLC Scanner 3, Camag Win CATS software, Camag twin-trough chamber and ultra sonicator were used during the study.

2.2. Preparation of working standard solutions

Working standard of Lomefloxacin Hydrochloride (50 mg) was weighed accurately and dissolved in 30 ml of 1:1 mixture of methanol and water since Lomefloxacin Hydrochloride is insoluble in organic solvents and made up to volume in a 50ml volumetric flask with the same solvent. This stock solution had a concentration of 1000mcg/ml Lomefloxacin Hydrochloride. From the above stock solution the final solution had a concentration of 5mcg/ml or 5ng/µl was prepared and this solution was used for application on HPTLC plate.

2.3. Preparation of sample solution

The contents of 20 tablets from Mahaquin and Lomaday were ground to a fine powder. Weight equivalent to 50 mg each of Lomefloxacin Hydrochloride was transferred to two 100ml conical flasks and dissolved in 30ml of 1:1 mixture of methanol and water and mixed gently,this solution was transferred into a 50ml volumetric flask through a Whatmann filter paper .no 41.The residue in the conical flask was extracted with further 10 ml of 1:1 methanol- water mixture and that was passed through the same filter paper the residue was extracted again with 5 ml of 1:1methanol water mixture and the final volume in each case was made up to 50 ml with 1:1 methanol water mixture . From the above stock solution the final solution had a concentration of 5mcg/ml or 5ng/µl was prepared.

2.4. Chromatographic conditions

The chromatographic estimation was performed using stationary phase, pre coated silica gel 60F 254aluminium sheets (20 × 10 cm, prewashed and dried in an oven at 50° for 5 min) and no chamber saturation. Standard solutions of Lomefloxacin Hydrochloride were applied to the plates, as 6 mm bands at 8 mm apart . The development distance was 20cm (development time 1 ½ hrs). After development the plates were removed from the chamber, dried by hot air blower and scanned through wavelength range of 200nm - 400nm and the spectrum shows an absorption maximum at 288 nm (Figure.1).

2.5. Selection of mobile phase

The mobile phase was selected by considering the adsorbent used and the polarity of Lomefloxacin hydrochloride. Organic acids can be separated by TLC as their ammonium salts of acid in Ethanol water. Several systems were tried by trial and error method .2-Propanol-Water –Ammonia 25 %(85:8.5:6.5) proved a good solvent system and gave R_F value of 0.15 (±0.05).

2.6. Development of calibration curve.

The programmer was set with a band width of 6mm and flow rate of 4 µl / second. Aliquots of 6, 8, 10, 12 and 14 µl l of standard solution of Lomefloxacin Hydrochloride were applied on the TLC plate. The TLC plate was dried, developed and analyzed photometrically as described earlier. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot. The method was validated by establishing linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak, as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. All the seven tracks were scanned and quantified at 285nm using a CAMAG TLC scanner. Figure.2 shows the densitogram of Lomefloxacin 40ng. The peak areas were noted. The data and the linearity plot obtained are shown in Table.1 and Figure.3 respectively.

2.7. Assay procedure for tablet solution

For the analysis of the marketed formulations, 10 µl each of extracted solutions of the marketed formulations were spotted on to the same plate to get a concentration of 50ng, followed by development and scanning. The spots were resolved into two peaks in the chromatogram of drug samples extracted from the marketed formulations.

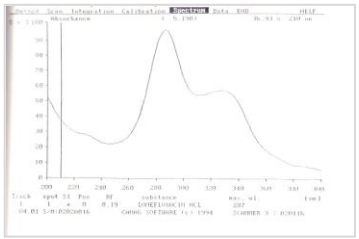

Figure.1.UV absorption spectrum of Lomefloxacin

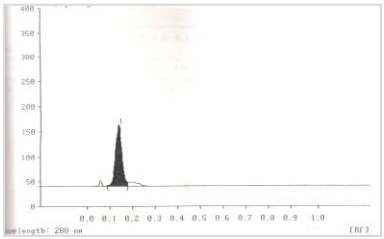

Figure .2.Densitogram of Lomefloxacin R.S

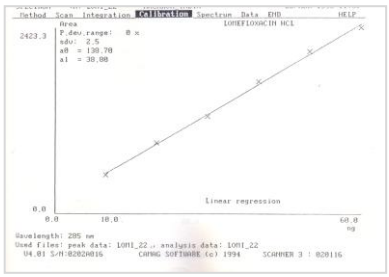

Figure.3.Linearity Curve of Lomefloxacin

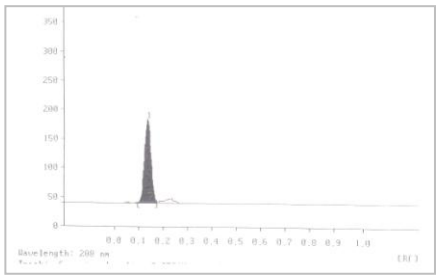

Figure 4.Densitogram of Mahaquin Peak area Vs Concentration

The content of the drug from Mahaquin and Lomaday were calculated from the peak areas recorded (shown in figure.3 and figure.4 respectively) and the data obtained is shown in table.2.

3. Method validation

The method was validated as per ICH guidelines [9] with respect to linearity, range, accuracy, specificity, precision, limit of detection, limit of quantitation and robustness.

3.1. Linearity and Range

Areas under curve (AUC) of five standard mixtures of different concentrations were determined and a calibration plot was obtained by plotting AUC area verses concentration equation. Linearity range was found to be in the range of 10-70 ng/ spot with a correlation coefficient of 0.9969. The LOD and LOQ were found to be 5 ng/spot and 10 ng/spot respectively.

3.2. Precision (Repeatability)

Precision of the method was tested by performing intra-day and inter-day studies. Intra-day precision was determined by analyzing standard solutions of Lomefloxacin hydrochloride in three times on the same day, and inter-day precision was determined by analysis of the same standards on three different days over a period of one week. The intra-day and inter-day relative standard deviations were in the ranges 0.74–1.09% and 1.29–1.46%.These low values indicated that the method is precise (Table.3)

3.3. Accuracy

The recovery study of the drug was carried out for accuracy parameters at multiple levels. Two sample weights of Lomefloxacin Hydrochloride marketed tablets equivalent to 10 mg was accurately weighed out and transferred to two 100 ml standard flask, added10 mg of Lomefloxacin Hydrochloride R.S to each flask and extracted by adding 20ml mobile phase initially, the volume was finally made up to 100 ml with mobile phase. The resulting solution had a concentration of 200µg/ml . Dilutions were made to get a concentration of 100ng/µl and recovery studies were performed. Percentage recovery was found to be within limits, as listed in Table.4.

3.4. Specificity

No interference was observed during analysis between drugs and excipients in tablet. Hence the method was found to be specific.

3.5. Robustness

The robustness of the method was studied for three different amounts (30, 40, and 60 ng per band) of Lomefloxacin Hydrochloride by determining the effects of volume of mobile phase used (±0.5%), time from sample application to chromatography (±20 min), and time between chromatography and scanning (±20 min). The RSD (%) of peak area was calculated for each change of conditions and found to be within the range stipulated by ICH guidelines (Table.5).

3.6. Ruggedness

In intra-day, inter-day variation and different analyst result of estimation by proposed method were found to be satisfactory which indicates ruggedness of the method.

Table.1 Calibration data for Lomefloxacin hydrochloride standard solution

Track No	*Volume of drug in µl*	*Concn.in ng*	*Peak Area*
1	6	30	965.4
2	8	40	1352.5
3	10	50	1807.8
4	12	60	2182.8
5	14	70	2574.1

Table.2.The assay results of the marketed formulations of Lomefloxacin

Sample	*Volume in µl*	*Average weight*	*Label claim*	*Area*	*% of label claim **	*%RSD*
Mahaquin	10	667.8 mg	400mg	1838.1	101.60	1.79
Lomaday	10	650.0mg	400mg	1821.8	100.42	0.86

**Each value is mean of three determinations*

Table.3. Precision data for the proposed HPTLC method

Concen.in ng/µl	*intra-day precision %RSD*	*Inter-day precision %RSD*
40	1.09	1.46
50	0.898	1.32
60	0.735	1.29

Table .4.Data for Recovery Study

Sample	*Vol. used in µl*	*Amount recovered In mg*	*Amount recovered*(mg) ± SD*	*% Recovery ± RSD*	*% RSD*
Mahaquin 10 mg + Lomefloxacin R.S 10 mg	3	10.36	10.36 ±0.20	101.4 ±1.36	1.36
	4	10.68	10.68 ±0.29	102.7 ±1.16	1.16
Lomaday 10 mg + Lomefloxacin R.S 10 mg	3	10.20	10.20 ± 0.16	100.80±0.72	0.72
	4	10.37	10.37 ± 0.22	101.48±1.59	1.59

** Each value is mean ± standard deviation of three determinations*

Table. 5. Results of the robustness testing of proposed method

Condition	RSD(%,n=3)
Amount of mobile phase (±0.5%)	1.02
Time from sample application to chromatography(±20 min)	1.67
Time from chromatography to scanning (±20 min)	1.15

4. Results and Discussion

As Lomefloxacin Hydrochloride is not freely soluble in water1:1 mixture of methanol and water was used. Tablet powder was extracted with this solvent.A variety of mobile phases were used for separation of Lomefloxacin Hydrochloride from other excipients present in formulations. The mixture 2-Propanol-water –Ammonia (86:8:6 %v/v/v) enabled satisfactory resolution of Lomefloxacin Hydrochloride with good peak shape and R_F values of 0.17 ± 0.05 (Figure.1.) The method was validated in accordance with ICH guidelines[9].The method was linear in the range 10–70ng per band Lomefloxacin Hydrochloride (with correlation coefficient 0.9969, n = 5).

The accuracy of the analysis was evaluated by the determination of recovery at three different concentrations of the drug in the dosage form. The results indicated that the method enables accurate estimation of the drugs in the tablet dosage form .The assay value for the marketed formulations were found to be within the limits, as listed in Table.2.

The low RSD value indicated the suitability of the method for routine analysis of Lomefloxacin Hydrochloride in pharmaceutical dosage forms.

5. Conclusion

It can be concluded that the developed HPTLC technique is simple, fast, precise, specific and the validation proved that the method is reproducible and selective for the analysis of Lomefloxacin Hydrochloride in bulk drug and tablet formulations.

Acknowledgement

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