TABLE 3: ANALYSIS OF ZIDOVUDINE AND LAMIVUDINE TABLETS

Name of company	Amount found mg/tablet ± SD.	%RSD.	Percent of assay
ZIDOVUDINE			
GLAXO	300.2±0.37	0.13	100.1
CIPLA	300.4±0.46	0.23	100.1
LAMIVUDINE			
GLAXO	150.4±0.86	0.34	100.3
CIPLA	150.7±0.85	0.61	100.4

A linear relationship was obtained for zidovudine 10-50 µg/ml. For lamivudine it was obtained at 10-30 µg/ml. Calibration curves could be represented by the following Eqns. $Y_{(zidovudine)}=0.0413X+0.0756$, (r=0.999) and $Y_{(lamivudine)}=0.0433X+0.0863$, (r=0.999). These equations were used for the determination of zidovudine and lamivudine from tablets.

ACKNOWLEDGEMENTS

The authors thank Dr. F. V. Manvi, Principal, K. L. E. S's College of Pharmacy, Belguam and Prof. A. D. Taranalli, Vice-Principal for the necessary facilities and encouragement.

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High Performance Thin Layer Chromatographic Method for Estimation of Moxifloxacin in Tablet Dosage Form.

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Accepted 6 February 2005

Revised 23 August 2004

Received 28 July 2003

A new simple, sensitive, specific and precise high performance thin layer chromatographic method has been developed for estimation of moxifloxacin in its tablet formulation (400 mg). In this method, standard solutions and sample solution of moxifloxacin were applied on precoated silica gel G60F_{254} TLC plate and developed using n- butanol:methanol:ammonia (4:4:2 v/v) as mobile phase.

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Moxifloxacin showed Rf value 0.50±0.03 and plate was scanned and quantified at 295 nm using Camag TLC Scanner 3. The method was validated in terms of linearity (400–1000 ng/spot), precision (intra-day variation 2.0 to 4.4%, inter-day variation 3.7 to 4.6%), accuracy (96.4 to 102.3%) and specificity. The limit of detection and limit of quantification for moxifloxacin were found to be 10 ng/spot and 50 ng/spot, respectively. The method is simple, sensitive, specific and precise and can be used for the routine quality control testing of marketed formulations.

Moxifloxacin is a broad-spectrum antibacterial drug, widely used in the treatment of community-acquired pneumonia, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis[1]. Various analytical methods that HPLC and capillary electrophoresis have been reported for the determination of moxifloxacin in biological fluid[2-6]. HPLC and spectrofluorimetry methods have also been reported for the estimation of moxifloxacin and its tablet formulations[7,8]. The present study describes development and validation of a simple, sensitive, specific and precise HPTLC method for the estimation of moxifloxacin from tablets.

Moxifloxacin working standard was procured as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad. Silica gel $G60F_{254}$ TLC plates (20×20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single component uncoated tablet formulations of moxifloxacin (400 mg) (formulation A- Moxif tablets, manufactured by Torrent Pharmaceutical Ltd, Ahmedabad and formulation B- Staxom tablets, manufactured by Ranbaxy laboratories Ltd, Goa) were purchased from a local pharmacy. n-Butanol, ammonia and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvents.

A Camag HPTLC system (Switzerland) comprising of Camag Linomat IV semiautomatic sample applicator, Camag TLC Scanner 3, Camag twin-trough chamber (10×10 cm), Camag CATS 4 software, Hamilton syringe (100 µl), Shimadzu libror AEG- 220 weighing balance, Sonicator (Frontline FS-4, Mumbai) were used in the study.

Moxifloxacin (10 mg) was weighed accurately and transferred to a 10 ml volumetric flask. It was dissolved in and diluted up to mark with methanol. The solution (1 ml) was diluted further to 10 ml with methanol. The final solution contained 100 µ g of moxifloxacin per ml of the solution. Ten tablets (each containing 400 mg moxifloxacin) were weighed and finely powdered. The powder equivalent to 10 mg of moxifloxacin was transferred to a volumetric flask and dissolved in 5 ml of methanol. The solution was sonicated for 10 min. The solution was filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with methanol. The filtrate (1 ml) was further diluted to 10 ml to have concentration of moxifloxacin equivalent to 100 µg/ml.

The chromatographic estimations were performed using following conditions: stationary phase, precoated silica gel $G60F_{254}$ aluminum sheets (20×10 cm, pre-washed with methanol and dried in air); mobile phase, n–butanol:methanol:ammonia (4:4:2 v/v); chamber saturation time, 30 min; Temperature, 29±3°; migration distance, 40 mm; wavelength of detection, 295 nm; slit dimensions, 3×0.45 mm; scanning speed, 5 mm/s. Following spotting parameters were used - band width, 4 mm; space between two bands, 4 mm and spraying rate, 10 sec/µl.

Six microlitres of standard solution of moxifloxacin (100 µg/ml) was applied along with standard on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 40 mm using mixture of n-butanol:methanol:ammonia (4:4:2 v/v) as mobile phase in a Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber, dried in air, scanned and quantified at 295 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using CATS 4 software incorporating the track optimization option.

Aliquots of 4, 5, 6, 7, 8 and 10 µl of standard solution of moxifloxacin (100 µg/ml) were spotted on precoated TLC plate. The TLC plate was developed and scanned as described under chromatographic separation. 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 area as well as repeatability of sample application. The limit of detection and limit of quantification were also determined.

Six microlitres of sample solution for formulation 1 and 2 (100 µg/ml) were applied on TLC plate, developed and scanned as described in chromatographic separa-

TABLE 1 : SUMMARY OF VALIDATION PARAMETERS OF MOXIFLOXACIN

Parameter	Result
Linearity range (ng/spot)	400-1000
Correlation co-efficient	0. 9956
Precision (% CV)	
Intra day (n=5)	2.0–4.4
Inter day (n=5)	3.7–4.6
Repeatability of sample application (n=7)	2.7
Repeatability of peak area (n=7)	0.34
% Recovery (n=3)	96.4–102.3
Limit of detection	10 ng/spot
Limit of quantification	50 ng/spot
Specificity	Specific

tion. Peak area were recorded and the amount of moxifloxacin present in formulations was estimated by using the calibration curve for moxifloxacin.

Literature survey indicated that various methods have been reported for analysis of moxifloxacin from its formulation and in biological fluid[2,8]. Most of them are HPLC[2-5,7], capillary electrophoresis[6] and spectrofluorimetry[8] which are sophisticated, expensive and time consuming. Moxifloxacin is not official in any pharmacopoeia. There is a need for a simple, sensitive and reproducible method for assay of moxifloxacin in their dosage form. Therefore, it was thought of interest to develop HPTLC a simple, versatile, speedy and cost effective method for determination of moxifloxacin in its tablet formulation.

The formulation was dissolved in methanol with sonication for 10 min to assure complete release of drug from the formulation matrix. The mixture of n-butanol:methanol:ammonia (4:4:2 v/v) resolved the peak of moxifloxacin (Rf =0.50±0.03) in the presence of other excipients in the formulations (fig. 1).

Linearity range for moxifloxacin was found to be in the range of 400 to 1000 ng/spot, with a correlation coefficient of 0.9956. The limit of detection and limit of quantification for moxifloxacin were found to be 10 ng/spot and 50 ng/spot, respectively.

The intra-day and inter-day precision (RSD) were determined for standard moxifloxacin (400-1000 ng/spot) for 5 times on the same day and over a period of week. The intra-day and inter-day coefficients of variation were found to be in the range of 2.0 to 4.4% and 3.7 to 4.6%, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (800 ng/spot) of moxifloxacin seven times without changing position of the plate and % CV for measurement of peak area was found to be 0.34%. Repeatability of sample application of the method was checked by spotting moxifloxacin (200 ng/spot) seven times on TLC plate (n=7) and % CV for peak area was found to be 2.7%. Both the % CV, for measurement of peak area and sample applications (less than 1% and 3%, respectively), ensuring proper functioning of HPTLC system. Accuracy of the method was evaluated by calculating recovery of moxifloxacin by standard addition method at 5 levels of the calibration curve (n=3). The percentage recovery was found to be 96.4 to 102.3 ensuring that the method is accurate.

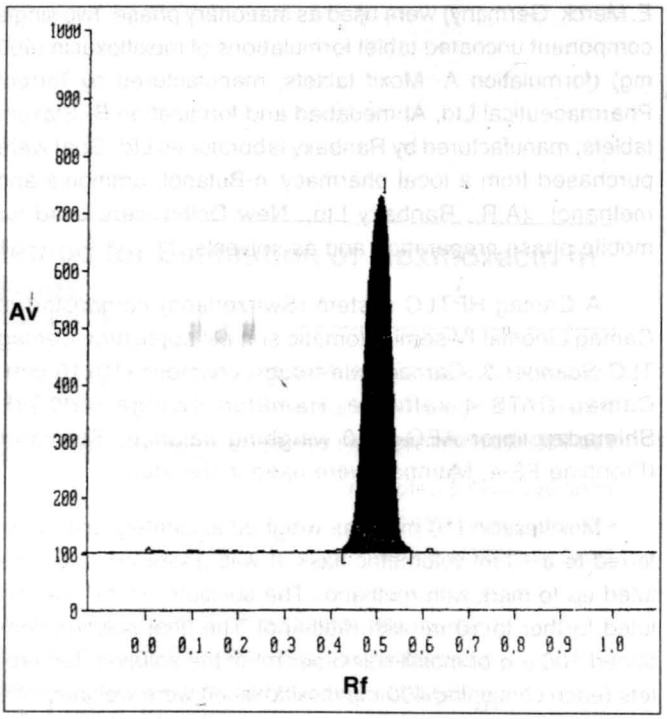

Fig. 1: Chromatogram of moxifloxacin from tablets

Chromatogram sample showing resolution of moxifloxacin peak (Rf= 0.50±0.03) from components of formulation matrix.

TABLE 2 : CONTENT OF MOXIFLOXACIN IN TABLET FORMULATION BY THE PROPOSED HPTLC METHOD.

Formulations	Labeled amount (mg)	Amount found (mg)*	% of drug found±SD*
1	400	401	100.3 ± 3.15
2	400	399	99.7 ± 1.64

* Average value±standard deviation of three determinations.

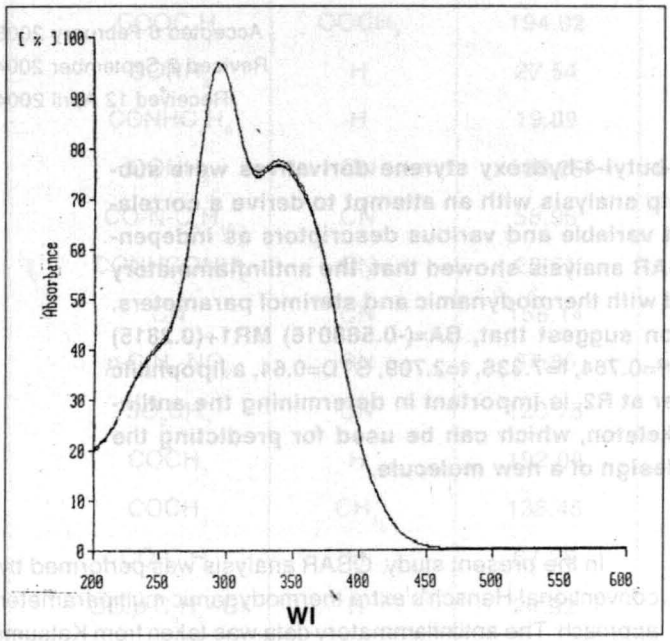

Fig. 2: Peak purity spectra of moxifloxacin

Peak purity spectra of sample moxifloxacin at peak start, peak apex and peak end.

Purity of the moxifloxacin peak from sample was determined by comparing the spectra at three different levels i.e. at peak start(S), peak apex (M) and peak end (E). Correlation between these three spectra indicated the purity of moxifloxacin peak (correlation, r(S,M)=0.9999, r(M,E)=0.9999, fig. 2). The spectrum of moxifloxacin extracted from tablet was also compared with spectrum of standard moxifloxacin, which showed good correlation (r=0.9993).

Different validation parameters for the proposed HPTLC method for determining moxifloxacin content are summarized in Table 1. This method was applied to determine the content of moxifloxacin in two different market samples of moxifloxacin tablets (Table 2). The result obtained is in agreement with the labeled value of moxifloxacin in dosage form. The results indicate that the proposed HPTLC method was found to be simple, specific, rapid, precise and accurate for assay of moxifloxacin in its formulations.

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