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A Simple and Sensitive HPTLC Method for Estimation of Gatifloxacin in Tablet Dosage Forms

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A simple and sensitive, HPTLC method has been developed for the quantitative estimation of gatifloxacin in its single component tablet formulations (400 mg). Gatifloxacin was chromatographed on silica Gel 60 F_{254} TLC plate using n-butanol:methanol:ammonia (6 M) (5:1:2 v/v) as mobile phase. Gatifloxacin showed Rf value 0.47±0.03 and scanned at 292 nm using a Camag TLC Scanner 3. The method was validated in terms of linearity (400–1200 ng/spot), precision (intra-day variation 1.3 to 3.2%, inter-day variation 3.9 to 5.0%), accuracy (93.3 to 99.4%) and specificity. The limit of detection and limit of quantification for gatifloxacin were found to be 10 ng/spot and 50 ng/spot, respectively. The developed method was successfully used for the assay of gatifloxacin tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Gatifloxacin is a broad-spectrum antibacterial drug, widely used in the treatment of community-acquired pneumonia, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, and complicated and uncomplicated urinary tract infections[1]. It acts intracellularly by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV[1]. Various analytical methods like HPLC and LC-MS have been reported for the estimation of gatifloxacin from its formulation and in biological fluids[2,3]. They are sophisticated, costly and time consuming. There is need of a simple, sensitive and precise method for assay of gatifloxacin in their dosage form. The present study describes development and validation of a simple, specific, sensitive, accurate and precise HPTLC method for the estimation of gatifloxacin in tablet formulation.

MATERIALS AND METHODS

Gatifloxacin working standard was procured as a gift sample. Silica gel 60 F_{254} TLC plates (20x20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single component uncoated tablet formulations of gatifloxacin (400 mg) (formulation A- Gatilox tablets, manufactured by Sun Pharma Ltd., Vadodara and formulation B- Tequin tablets, manufactured by Cadila Healthcare Ltd., Ahmedabad) were purchased from a local pharmacy. n-Butanol, ammonia (6 M) and methanol (AR, 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 (10x10 cm), Camag CATS 4 software, Hamilton syringe (100 µl), Shimadzu libror AEG-220 weighing balance, Sonicator (Frontline FS-4, Mumbai) were used during the study.

Preparation of standard gatifloxacin solution and samples:

Gatifloxacin (10 mg) was weighed accurately and transferred to a 10 ml volumetric flask. It was dissolved in and diluted up to mark with methanol. One millilitre was further

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

HPTLC method and chromatographic condition:

The chromatographic estimations were performed using following conditions; stationary phase, precoated silica gel 60 F_{254} aluminum sheets (20×10 cm) (pre-washed with methanol. and dry in air); mobile phase, n–butanol:methanol:ammonia (5:1:2 v/v); chamber saturation time, 30 min; Temperature, 29±4°; migration distance, 40 mm; wavelength of detection, 292 nm; slit dimensions, 4×0.1 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.

Chromatographic separation:

Six microlitres of standard solution of gatifloxacin (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 40 mm at constant temperature using mixture of n-butanol:methanol:ammonia (5:1:2 v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 292 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using CATS 4 software incorporating the track optimization option.

Preparation of calibration curve:

Aliquots of 4, 6, 8, 10, and 12 µl of standard solution of gatifloxacin (100 µg/ml) were spotted on precoated TLC plate, using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

Validation of method:

The method was validated in terms of 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.

Quantification of gatifloxacin in tablet formulation:

Eight microlitres of sample solution for formulation A and B (100 µg/ml) were applied on the TLC plate, developed and scanned as described in chromatographic separation. The amount of gatifloxacin present in the sample solution was determined by fitting area values of peak corresponding to gatifloxacin into the equation of line representing calibration curve of gatifloxacin.

RESULTS AND DISCUSSION

Gatifloxacin is soluble in methanol; therefore methanol was selected as solvent. 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 (5:1:2 v/v) could resolve gatifloxacin spot with better peak shape. Combination of n-butanol and methanol offered optimum migration (Rf= 0.47±0.03) and resolution of gatifloxacin from other components of formulation matrix (fig. 1). On the other hand, ammonia solution helped in sharpening of the peak. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution.

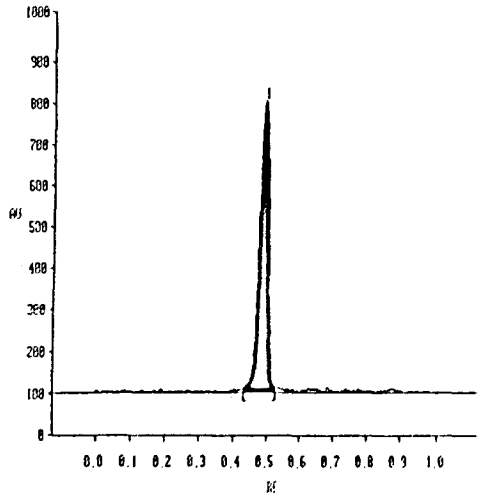

Fig. 1: Chromatogram of gatifloxacin from tablets.

Chromatogram of the sample showing resolution of gatifloxacin peak (800 ng/spot, R_f=0.47) from components of formulation matrix.

Linearity range for gatifloxacin was found to be in the range of 400 to 1200 ng/spot, with a correlation coefficient of 0.9976. The average linear regression equation was represented as Y=8.4346X+16032, where X=concentration of gatifloxacin and Y=peak area. The limit of detection and limit of quantification for gatifloxacin were found to be 10 ng/spot and 50 ng/spot, respectively.

The intra-day precision (RSD) was determined for standard gatifloxacin (400-1200 ng/spot) for 3 times on the same day. The inter-day precision (RSD) was calculated for standard gatifloxacin (400-1200 ng/spot) for 5 times over a period of one week. The intra-day and inter-day coefficients of variation were found to be in the range of 1.3 to 3.2% and 3.9 to 5.0%, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (1200 ng/spot) of gatifloxacin seven times without changing position of the plate and % CV for measurement of peak area was found to be 0.29%. Repeatability of the method was checked by spotting 12 µl of gatifloxacin standard solution seven times on TLC plate (n=7) and % CV for peak area was found to be 0.81%. 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 method was evaluated by calculating recovery of gatifloxacin by standard addition method at 5 levels of the calibration curve (n=3). The percentage recovery was found to be 93.3 to 99.4% ensuring that the method is accurate. The method is found to be specific for gatifloxacin. The purity of the gatifloxacin peak 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 gatifloxacin peak (correlation, r(S,M)=0.9998, r(M,E)=0.9997, fig. 2). The spectrum of gatifloxacin extracted from tablet was also compared with spectrum of standard gatifloxacin, which showed correlation of 0.9993.

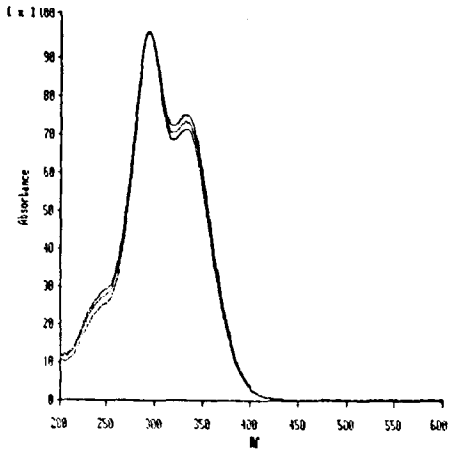

Fig. 2: Peak purity spectra of gatifloxacin.

Peak purity spectra of standard gatifloxacin (600 ng/spot) at peak start, peak apex and peak end.

Different validation parameters for the proposed HPTLC method for determining gatifloxacin content are summarized in Table 1. This method was applied to determine the content of gatifloxacin in two different market samples of single component gatifloxacin tablets. The content and percentage of gatifloxacin in two different market samples were found to be 413.2 mg, 103.3±3.87% and 402.4 mg, 100.6±3.06%, respectively (n=3). The results indicate that the proposed HPTLC method was found to be simple, specific, rapid, precise and accurate for estimation of gatifloxacin in its formulations.

TABLE 1: SUMMARY OF VALIDATION PARAMETERS OF GATIFLOXACIN

Parameter	Results
Linearity range (ng/spot)	400-1200
Correlation co-efficient	0. 9976
Precision (% CV)	
Intra day (n=3)	1.3-3.2
Inter day (n=5)	3.9-5.0
Repeatability of sample application (n=7)	0.81
Repeatability of peak area (n=7)	0.29
% Recovery (n=3)	93.3-99.4
Limit of detection (ng/spot)	10
Limit of quantification (ng/spot)	50
Specificity	Specific

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