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Development and Validation of TLC Densitometric Method for Gatifloxacin in Pharmaceutical Formulations

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Abstract: A simple, rapid and accurate High-performance thin-layer chromatography (HPTLC) method has been established and validated for the simultaneous determination of Gatifloxacin in tablets. The method is based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 288 nm. Validation was carried out in compliance with International Conference on Harmonization guidelines. The method employed thin-layer chromatography aluminium plates pre-coated with silica gel 60F-$_{254}$ as stationary phase. The solvent system consisted of toluene: acetic acid: Triethylamine the ratio of (4.0:2.5: 0.5 v/v) as a mobile phase. This solvent system was found to give compact spots for Gatifloxacin with R_f value 0.46. Ciprofloxacin used as internal standard. Method was successively applied to tablet formulation. The method was validated for precision, accuracy and robustness. Pure drug was subjected to acid and alkali hydrolysis, oxidation, photo degradation, dry heat and wet heat treatment. The drug underwent degradation under acidic, basic, oxidative and wet heat conditions. The method was validated in accordance with the requirements of ICH guidelines.

Key words: Gatifloxacin, Ciprofloxacin, HPTLC, stability.

Introduction:

Gatifloxacin is 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl) 4-oxo-3-quinoline carboxilic acid[1].Literature survey reveals that few, Spectrophotometric, HPLC and HPTLC [2-7] ,stability indicating HPTLC [8,9] for the estimation of gatifloxacin alone or in combination with other agents are reported. Author of the article and his research team has developed a HPTLC Method development different pharmaceutical dosage form[10-14].The International Conference on Harmonization (ICH) guidelines entitled as 'Stability testing of new drug substances and products' recommends the stress testing of active pharmaceutical ingredient (API) to elucidate its stability. Susceptibility to oxidation, hydrolytic and photolytic stability is some of the recommended tests [15]. An ideal stability- indicating method is one that quantifies the drug and resolves its degradation products. HPTLC method is becoming a routine analysis technique due to some advantages over other methods; the aim of this work is to develop an accurate, specific and reproducible stability-indicating method for determination of gatifloxacin in presence of its degradation products and related impurities as per ICH guidelines.

Experimental

HPTLC method and chromatographic conditions:
The chromatography estimation was performed using precoated silica gel 60 F_{254} aluminum sheets (10 x 10 cm, E. Merck) and mixture of toluene, acetic acid , Triethylamine (4.0 :2.5 : 0.5 v/v)as mobile phase. The chamber has saturated for 25 min and the plate developing up to 5 cm. The plates were pretreated with methanol and activated at 60 °C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was employed and the bands were separated by a distance of 8 mm. The monochromatic bandwidth was set at 35 nm, each track was scanned thrice and baseline correction was used.Densitometric scanning at 288 nm was then performed with a Camag TLC Scanner III in absorbance mode operated by Win CATS software. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm. The slit dimension was kept 6 × 0.45 mm and 15 mm/s scanning speed were employed. All chemicals and reagents used were of analytical grade and were purchased from Merck chemicals, India.

Calibration curve of Gatifloxacin
Standard solutions of Gatifloxacin (10 µl each) were applied in triplicate on TLC plate. The plate was developed in solvent system composed of toluene: acetic acid: Triethylamine the ratio of (4.0:2.5: 0.5 v/v) up to distance of 5 cm. Composed of development, the plates were dried in hot air and scanned at 288 nm. The peak areas were recorded. Calibration curve of Gatifloxacin was obtained by plotting peak area *vs* concentration of Gatifloxacin applied.

Method validation

Precision
The repeatability of sample application and measurement of peak area for active compound were expressed in terms of percentage relative standard deviation (%RSD) and found to be less than 2%. System intra-day repeatability was determined by five replicate applications and five times measurement of a standard Gatifloxacin solution at the analytical concentration of 400 ng/spot.

Robustness
Mobile phases having different composition of toluene: acetic acid: Triethylamine the ratio of (4.0:2.5:0.5 v/v) and (2.0:3.5: 1.0 v/v/v/) were tried and chromatograms were run. Robustness of the method was done at three different concentration levels of 200,300 and 400 ng/spot.

Linearity
Accurate quantities from working standard solutions (0.5, 2.0, 4.5, 5.5, 7, 8 and 10 µg/ml) were applied to the TLC plate to give bands containing 200–400 ng spot $^{-1}$ of Gatifloxacin. Each amount was applied five times and the plate was developed, using the previously described optimized mobile phase, and scanned. The calibration curves were constructed by plotting peak areas versus concentrations.

Accuracy
Accuracy of the method was determined by standard addition method in which the known amount of standard Gatifloxacin solutions were added to pre analyzed tablet solution. These amounts corresponded to 50, 100 and 150 % of the amounts claimed on the label. The amounts of Gatifloxacin were estimated by applying these values to the regression equation of the calibration curve.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
In order to determine LOD and LOQ, concentrations in the lower part of the linear range of the calibration curve were used. Stock solution of Gatifloxacin (0.10 mg mL^{-1}) was prepared and different volume of stock solution in the range 200 to 400 ng spot $^{-1}$ were spotted in triplicate. The amount Gatifloxacin by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.) / b and quantification limit was calculated by (10×A.S.D.) / b, where "b" corresponds to the slope obtained in the linearity study of method.

Recovery studies
The analysed samples were spiked with extra 100,120 and 150% of the standard Gatifloxacin and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of drug at different levels in formulations.

Quantification of Gatifloxacin in Tablet
To determine the content of Gatifloxacin in tablet (label claim: 10 mg/tablet), the mean weight of twenty Gatifloxacin tablets was determined and then the tablets were finely powdered. Weight of powder equivalent to content in a tablet was transferred to a 100 ml volumetric flask containing 50 ml distilled water, sonicated for 30 min and diluted to 100 ml again with distilled water. From the resulting solution, supernatant was collected and analyzed for drug content. One µl of the filtered solution (200 ng/spot)

was applied on the TLC plate followed by development and scanning as per stated procedure.

Forced Degradation Studies of Gatifloxacin

A stock solution containing 10 mg Gatifloxacin in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of Gatifloxacin after application (400 ngspot-^{1}) of five replicates was obtained. In order to study the degradation products of Gatifloxacin using HPTLC method most of the study was carried out by single development of TLC plate in order to prevent the movement of the non-polar degradates to extreme end of the plate. The plate was developed and scanned in above established chromatographic conditions.

Acid and Base Induced Degradation

Acid decomposition studies were performed by refluxing the solution of drug in 1 N HCl and 1 N NaOH. These solutions were kept for 8 h and 6 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 mL of above solutions was taken and neutralized, then diluted up to 10 mL with methanol. The forced degradation in acidic and basic media was allowed to occur in dark in order to exclude the possible degradative effect of light. 1 µL each of the resultant solutions (200 ng/spot) were applied on TLC plate and the chromatograms were run as per stated procedure.

Hydrogen peroxide-induced degradation

To study hydrogen peroxide induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature for 12 h. Subsequently drug was exposed to 30% hydrogen peroxide at room temperature for a period of 24 h then heated in boiling water bath for 10 min to completely remove the excess of hydrogen peroxide.

Dry heat and wet heat degradation product

The powdered drug was stored at 65^{0}C for 24 h under dry heat condition showed significant degradation. The degraded product was resolved from the standard. In all degradation studies, the average peak areas of Gatifloxacin after application (400 ng spot^{-1}) of three replicates were obtained.

Photochemical degradation product

The photochemical stability of the drug was studied by exposing the stock solution (1 mg mL^{-1}) as well as solid drug to direct sunlight for 10 days on a wooden plank and kept on terrace. The solution was kept in the sun light for 10 h.

Neutral Hydrolysis

To study degradation behaviour of drug in neutral condition drug was dissolved in water and solution was refluxed at 55°C for 6 days and subsequently for 15 days.

Table 1: Intra-day and inter-day precision of RP-TLC method

Amount ng spot -1	Amount found*	S. D.	%R. S. D
Intra-day			
200	199.77	1.95	0.176
300	300.04	2.05	0.438
400	400.09	1.17	0.427
Inter-day			
200	194.94	2.57	0.321
300	296.21	4.93	0.768
400	391.95	2.01	0.968

*mean of three determinations

Table 2: Summary of Validation Parameters of Proposed HPTLC method

Parameter	Value
R_f (SD)	0.46
Linearity and range (ng\spot)	200-400ng/spot
Limit of detection(ng\spot)	40 ng/spot
Limit of quantification (ng\spot)	88 ng/spot
% Accuracy ± SD[a] (n=6)	99.98 % ± 0.054
Precision (% RSD[b])	
(a) Repeatability of sample application (n=5)	0.805 %
(b) Repeatability of sample measurement (n=5)	0.363 %
Intraday (%RSD)	0.954-1.464 %
Inter day (%RSD)	0.480– 0.792 %
% Assay ± SD[a] (n=6)	99.92 % ± 0.043
LOD[a]	0.216
LOQ[b]	0.329
Robustness	Robust

Table 3-Recovery Studies of Gatifloxacin

Gatifloxacin			
Label Claimed(mg)	**Excess drug added to the analyte (%)**	**Amount recovered(ng)**	**% recovery**
10	0	10.00	100.00
	80	9.99	99.983
	100	10.15	100.16
	120	10.29	100.09

[a]SD = Standard deviation, [b]RSD = Relative standard deviation

Table-4 Summary of degradation products of Gatifloxacin under different stress conditions

Sample exposure condition	Number of degradation products (Rf values)	Gatifloxacin remained (ng/400 ng)	Recovery (%)
1N HCl RT[a]	0.35	9.75	97.22
1N NaOH RT[a]	0.53	9.55	95.50
Oxidation(H_2O_2,10% v/v),	0.69	9.87	98.70
Dry heat, 30h , 65^{0}C	0.19	8.98	89.98
Photolytic Condition	0.26	9.11	91.11

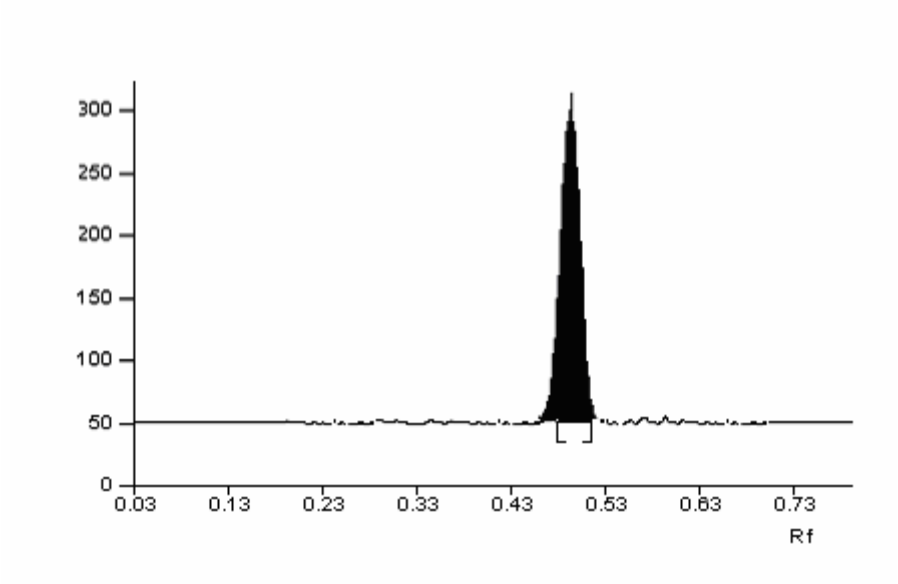

Fig. 1. A typical densitogram of standard Gatifloxacin with R_f value 0.46

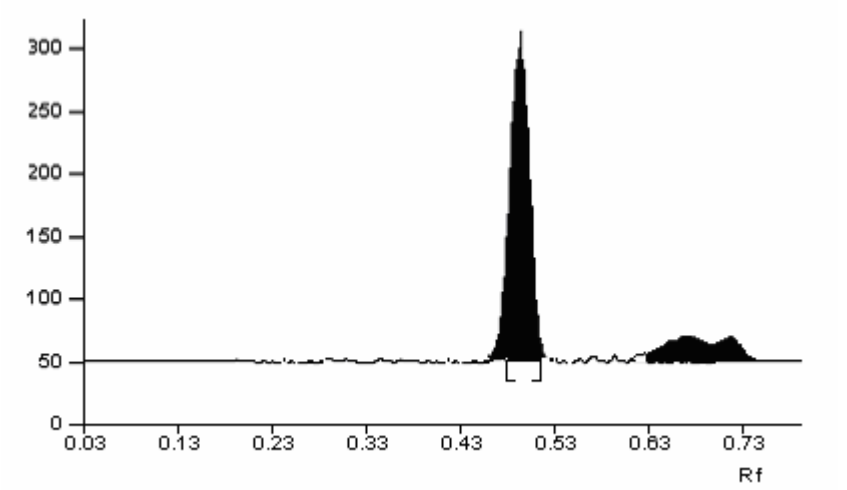

Fig.2 Chromatogram obtained after oxidative degradation of Gatifloxacin

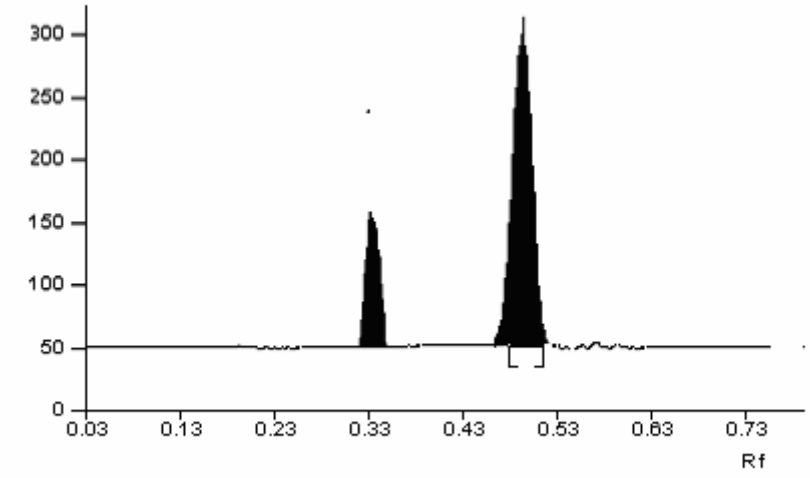

Fig.3 Chromatogram obtained after 1N HCl degradation of Gatifloxacin

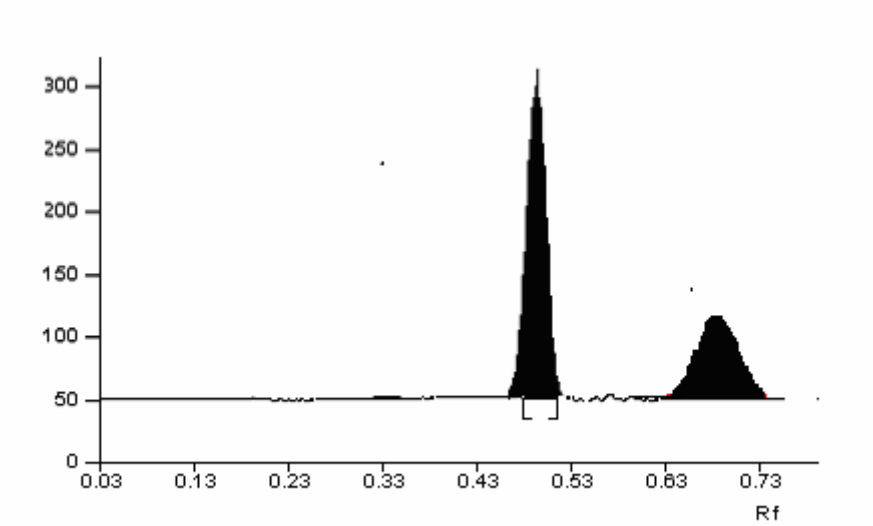

Fig.4 Chromatogram obtained after 1N NaOH degradation of Gatifloxacin

Results and discussion

The TLC procedure was optimized with a view to develop a stability-indicating assay method. Both the pure drug and the degraded drug solution were spotted on the TLC plates and run in different solvent systems. The mobile phase, which was used, for the estimation of Gatifloxacin toluene: acetic acid: Triethylamine the ratio of (4.0:2.5: 0.5 v/v) gave good resolution with R_f value of 0.46 for Gatifloxacin and degraded product gave a sharp and symmetrical peak. Finally this mobile phase was optimized to achieve good peak symmetry and better separation of Gatifloxacin from degradation peaks. The spot appeared to be more compact with a more symmetrical peak shape when TLC plates were pre-treated with methanol and activated at 110ºC for 5min. Well-defined standard spots along with its degradation products were obtained when the chamber saturation time was optimized to 30min at 25ºC temperature. The calibration plot was linear over a concentration range of 200–400 ng per spot. A good linear relationship was observed over this range (r^2= 0.9989 ± 0.037). Repeatability of sample application and measurement of peak area was expressed as RSD and were 0.805 % and 0.363 % for five replicate determinations. The low values of RSD indicate that the proposed method is repeatable. The RSD value obtained for intra-day and inter-day variation were 0.954-1.464 % and 0.480– 0.792 % respectively is low which indicates that proposed method is precise. RSD of peak areas during robustness studies were calculated for changes in parameters and were less than 2% which indicates that method is robust and reproducible. LOD and LOQ values were found to be 40 and 88 ng per spot, respectively, and pointed towards adequate sensitivity of the method. A single band was observed in samples extracted from tablets and there was no interference from the excipients which might have present in the tablets. The drug content was found to be 99.90 % with standard deviation 0.298. It was therefore inferred that degradation of Gatifloxacin had not occurred in the marketed formulation analyzed by this method. There was no indication of degradation in solutions of Gatifloxacin as revealed by peak purity data and from the value of RSD (< 2%) for peak areas of bands of solution stored at different times. The solution was found to be stable at ambient temperature for 40 h and no unknown peaks were observed.

Conclusion

The developed HPTLC procedure was precise, specific and accurate. Separation of Gatifloxacin from degradation products confirmed stability indicating properties of this method. Statistical analysis indicated that the method was reproducible and selective for the analysis of Gatifloxacin in bulk drug and in tablets without interference from excipients.

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