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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF GATIFLOXACIN IN PHARMACEUTICAL FORMULATIONS

Syeda kulsum, Mohd. Mudassir Hussain and Abdul Mannan*
MRM College of Pharmacy, Ibrahimpatnam, Hyderabad, India.
Dr. Abdul Mannan, Professor, MRM College of Pharmacy, Ibrahimpatnam, Hyderabad, India.
Mohd. Mudassir Hussain, Assitant Professor, MRM College of Pharmacy, Ibrahimpatnam, Hyderabad, India.
Email:syedakulsum@gmail.com

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Abstract:

In the present analytical project, an attempt has been made to develop a simple, economical and reliable liquid chromatographic method for the determination of Gatifloxacin belonging to the class of organic compounds known as quinoline carboxylic acids. It is a synthetic broad spectrum fluoroquinolone antibiotic obtained from nalidixic acid, used to treat a wide range of infectious diseases. The chromatographic separation was performed on a C-18 analytical column, using isocratic elution containing pH 2.8 buffer (using orthophosphoric acid) and methanol in ratio (60:40). Several absorption spectra were obtained for each peak using a PDA detector. The effluents were monitored at 293nm and flow rate was fixed as 1.0 ml / min. The retention time was 3.148 min. The linearity was in the range of 72-218 μg / ml. This method was validated as per the required ICH guidelines. Statistical analysis data proves that the method is precise, reproducible and selective for the estimation of the Gatifloxacin in both small and bulk formulations. Further this method is also useful in finding the impurities during the stability studies of the different dosage form formulations.

Keywords: RP-HPLC, Gatifloxacin, Validation Stability

Introduction:

Gatifloxacin (GTX) is a synthetic broad spectrum fluoroquinolone antibiotic obtained from nalidixic acid, used to treat a wide range of infectious diseases[1].It belongs to the class of organic compounds known as quinoline carboxylic acids. These are quinolines in which the quinoline ring system is substituted by a carboxyl group at one or more positions. Published structure–activity data show that the presence of fluorine atom (F) at C6 position broadens their activity spectrum against both Gram-negative and Gram-positive pathogens[2]. It is chemically 1-cyclopropyl-6-flouro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid[3].

Gatifloxacin is administered topically to the eye. Gatifloxacin is widely and rapidly distributed to systemic tissues; most target tissues have higher concentrations relative to serum. There were few methods developed previously to estimate gatifloxacin by TLC, UV [4,5,6,7 and 8.]

The objective of the work was to develop simple, accurate, precise and economic RP-HPLC method with lesser run time to estimate the Gatifloxacin in bulk and pharmaceutical dosage forms.

Materials and methods:

The liquid chromatographic system consisted of following components. A Shimadzu HPLC model 2695, UV detector 2487 and Pump, variable wavelength PDA detector and Hamilton syringe (50 µL).

Chromatographic analysis was performed using empower software on an Agilent (4.6×150mm) 5µ column. The mobile phase consisting of buffer and methanol (60:40% v/v). The optimized chromatographic conditions are summarized in Table 1 and gatifloxacin structure is seen in figure-1.

Development and Validation of Stability indicating HPLC method for Gatifloxacin

A stability indicating method for the determination of Gatifloxacin by RP-HPLC was developed and validated according to USP and ICH guidelines. The solubility of the drug was determined. The appropriate wavelength in UV region has been selected for the measurement in the proposed method.

In HPLC method, the conditions were optimized to obtain an adequate elution of Gatifloxacin. Mobile phase, column selection, wavelength selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor) and run time. The mobile phase was with pH 2.8 buffer and methanol with flow rate of 1.0 ml /min was found to be robust. The optimum wavelength for detection was 293nm and a run time of 8min at which better detector for the drug along with no interference was obtained. The standard chromatograms were taken for the proposed method and various system suitable parameters were recorded. Forced degradation studies were conducted to demonstrate that the method was stability indicating. Robustness is carried out to show that method is robust.

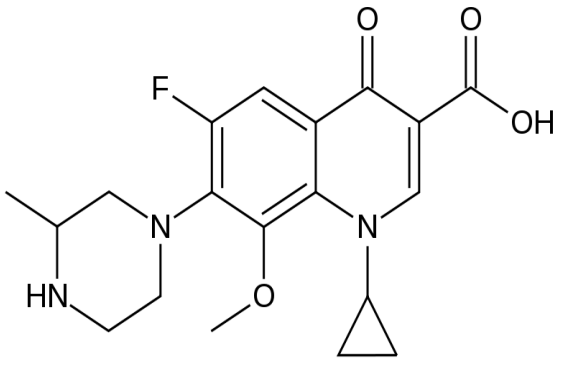


Fig no. 1: structure of gatifloxacin.

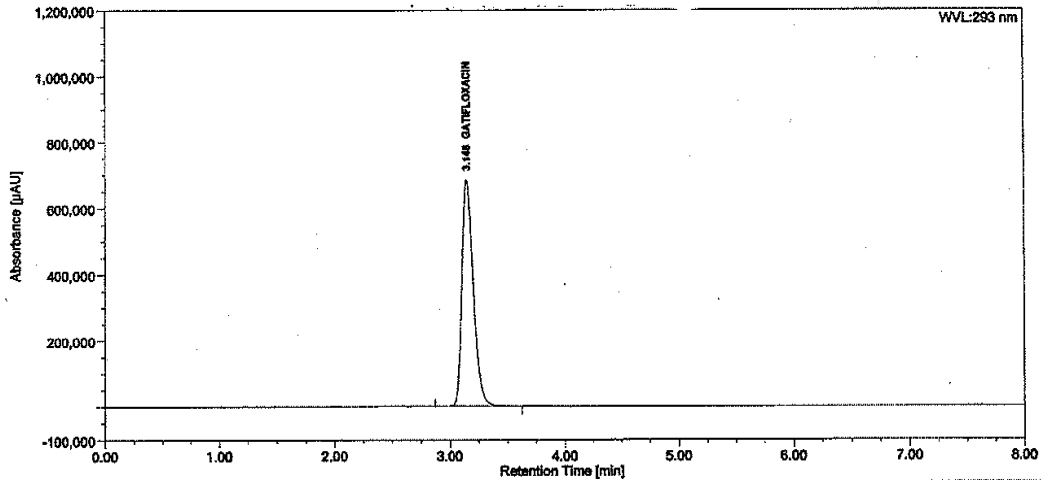


Fig No. 2: Chromatogram for Gatifloxacin.

Name	Retention time	Area	USP Tailing	USP Plate count
Gatifloxacin	3.148 min	500458	1.35	3542

From the above chromatogram it was concluded that it is an optimised method all the system suitability parameters were passed like theoretical plates were more than 2000, tailing factor is less than 2.0 and RSD is not more than 2.0% This method is used for the regular analysis, stability samples and also for Validation

Method Validation

This analytical method validation for Assay of Gatifloxacin ophthalmic solution is carried out as below.

System Suitability:

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard solutions. It was observed that the method complies with the system suitability parameters. Hence it was concluded that the system suitability parameter met the requirement of method validation.

Table No. 1: Results of System Suitability parameters.

SNO	System Suitability Parameters	Proposed Acceptance Criteria	Results
1	Tailing factor	NMT 2.0.	1.17
2	Theoretical plates	NLT 2000	4219
3	%RSD	NMT 2.0%	0.1%

Specificity:

Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present, such as impurities, degradation products and matrix components. The diluent peaks were not interfered with Gatifloxacin peak.

Specificity by Forced Degradation Studies:

Forced degradation of Gatifloxacin Ophthalmic Solution shall be carried out, to confirm that during stability study or throughout the shelf life, any degradation product if found should not interfere with the main peak of Gatifloxacin. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, water hydrolysis, photolytic and dry heat) from the results it was concluded that the Gatifloxacin peak is found to be degraded less in acidic stressed condition and from the above data, it can be concluded that the Gatifloxacin Peak is found to be pure.

All the unknown impurity/Degradation products were well separated with Gatifloxacin peak.

For the stressed placebo, no interference at the retention time of Gatifloxacin peak.

Peak purity factor for Gatifloxacin peak was more than 990 in all stressed condition.

Table No. 2: Results for Specificity by Degradatoin Studies.

Stress condition	**As such**	**0.1 N NaOH**	**1.0 N NaOH**	**O.1 N HCl**	**1.0 N HCl**	**3.0% w/v Peroxide**	**Nuetral**	**Thermal**	**Sun light**	**UV light**
Peak purity	P	P	P	P	P	P	P	P	P	P
Peak purity factor	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Compound name	% Assay									
Gatifloxacin	102.6	93.4	83.0	90.9	78.0	68.5	100.7	85.7	102.0	101.6

Peak purity: 'P' indicates Gatifloxacin peak is pure which confirmed by Diode array detector and Agilent Chemstationsoftware.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements.

A) System Precision:

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area response of six determinations should be measured and calculate relative standard deviation. The retention time & area response are consistent as evident from relative standard deviation and the system precision parameters meets the requirement of validation.

Table No. 3: Results of System Precision.

SNO	System Suitability Parameters	Proposed Acceptance Criteria	Results
1	The % RSD of the Retention timeforGatifloxacin peak obtained from 6 injections of Standard preparation	NMT 1.0%	0.1%
2	The % RSD of the Area responseforGatifloxacin peak obtained from 6 injections of Standard preparation	NMT 2.0%.	0.1%

B) Method Precision:

In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch.

Analyzed the sample of Gatifloxacin Ophthlmic Solution six times of a same batch as per analytical procedure. Calculated the % of Assay with respect to the standard solution.It was concluded that the method is precise.

Table No. 4: Results of Method Precision.

SNO	System Suitability Parameters	Proposed Acceptance Criteria	Results
1	The results should be with in specification limit.	The results should be with in specification limit.	The results were with in specification limit.
2	The % RSD calculated on 6 determinations	NMT 2.0%	0.3%

C) Intermediate Precision:

The intermediate precision should be carried out to ensure that the analytical results will remain unaffected with change in instrument, column and day. It was concluded that the method is precise.

Table No. 5: Results of Intermediate Precision.

SNO	System Suitability Parameters	Proposed Acceptance Criteria	Results
1	The % RSD calculated on 6 determinations	NMT 2.0%	0.2%
2	The % RSD calculated on 12 determinations (Method precision & intermediate precision)	NMT 2.0%.	0.4%

Stability in Analytical Solution:

The stability in analytical solution was evaluated by injecting the Standard solution and Sample solution at regular interval. The standard solution was stable for 27 Hrs, sample solution is stable up to 25 Hrs at room temperature (25°C)

Table No. 6: Results for Stability of Analytical Solution.

S.No	Validation parameter	Acceptance criteria	Results
1	Stability in analytical solution	The % Difference of Area Response for theGatifloxacin peak in Standard solution and sample solution should be within ± 2.0% from initial area after specified period.	Standard is stable upto 27 hours (The % difference is 0.1) and the sample is stable upto 25 hours(The % difference is 0.4) at room temperature 25

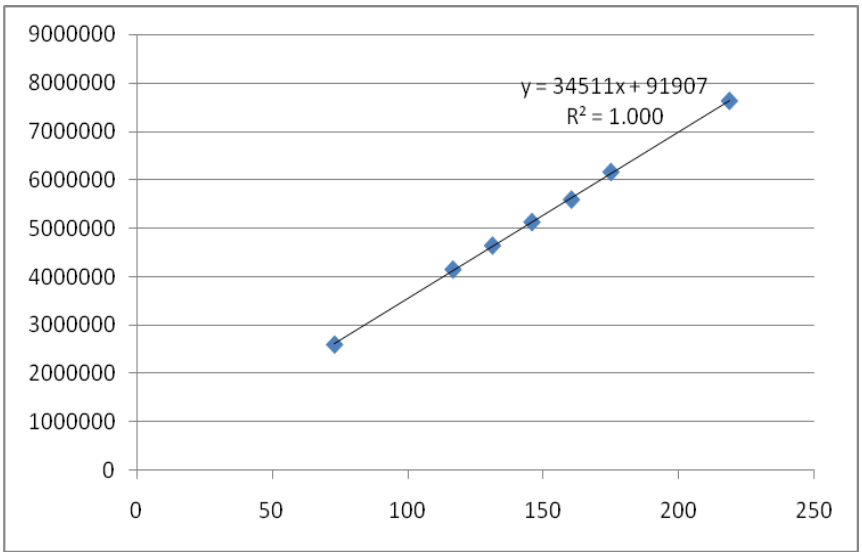
Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The response of Gatifloxacin is linear between 50% to 150% level of working concentration. The correlation & regression coefficient were more than and equal to 0.998. Moreover, the value of intercept is within ±2% of the area response at 100% level.

TableNo.7: Concentrations in Linearity.

Level	Concentration inµg/ml	Peak Area(µV×sec)
1	72.900	2588876.147
2	116.6400	4141537.610
3	131.2200	4636438.741
4	145.8000	5122050.147
5	160.3800	5588594.859

6	174.9600	158222.524
7	218.700	7629869.071
Correlation Coefficient		1.000
Slope		34511.306
Intercept		91907.158
% Intercept		1.8%



TableNo.3: Linearity of Gatifloxacin.

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value) and it was concluded that the recovery is well within the limit. Hence the method is accurate.

TableNo.8: Results of Accuracy.

S.No	Proposed Acceptance Criteria	Levels	% Recovery
1	Mean % recovery at each level should be between 98.0 % and 102.0 %.	50%	101.4%
		100%	99.6%
		150%	98.9%

Range:

The range of analytical method is the interval between the upper and lower levels of analyte that has been demonstrated to be determined with a suitable accuracy and linearity. The range of the method is from 50% to 150% of target concentration for Gatifloxacin.

TableNo.9: Linearity range & Accuracy range.

Level (Conc. in % about)	**Linearity range**	**Accuracy range**
50	2588876.147	2623000.993
100	5122050.147	5153433.721
150	7629869.071	7671335.387
Correlation coefficient	1.000	1.000
RSD for all Levels	----	1.2%

Robustness:

Robustness parameters like Change in column temperature ± 5^0C, Change in flow rate ± 0.2 mL/min, Change in Organic phase ± 2.0% and Change in pH± 0.2% was deliberately done. From the results it is concluded that the method is robust.

Table No.10: Results of Robustness.

Acceptance Criteria	The RSD for Gatifloxacin peak in standard solution should be not more than 2.0%	The Theoretical plates of Gatifloxacin peak should be not less than 2.0	Tailing factor
Original conditions	0.1%	4219	1.17
Decrease in Flow rate (1.3 mL/min)	0.0%	3269	1.10
Increase in Flow rate (1.7mL/min)	0.0%	3871	1.13
Decrease in Column temp. (20°C)	0.0%	3624	1.12
Increase in Column temp. (30°C)	0.0%	2969	1.08
Decrease in Organic phase (-2.0%)	0.0%	2821	1.50
Increase in Organic phase (+2.0%)	0.1%	2807	1.57
Decrease in pH (-0.2 % - 6.6 unit)	0.1%	3493	1.37
Increase in pH (+0.2 %- 7.0 unit)	0.1%	3743	1.21

Limit of Detection & **Limit of Quantitation**

From the intercept, slope and residual standard deviation calculated from the linearity curve limit of detection(0.01) and limit of Quantitation(0.04) was also determined which indicated that the method is sensitive.

Table No. 11: LOD & LOQ.

Sr. No.	Gatifloxacin	
	Concentration (in µg/ml)	**Peak Area(mv.sec)**
1	0.0060	873.637
2	0.0120	1896.727
3	0.0200	7301.189
4	0.0360	11643.719
5	0.0600	17901.444
SD	1322.54	
Slope	320567.94	
LOD (ppm)	0.01	
LOQ (ppm)	0.04	

Conclusion

As shown in the results a simple and effective reverse liquid choromatography method was developed and validated as per the standard ICH guidelines. A good linear relationship was observed in the concentration ranges of 72-218µg/ml. The correlation coefficient was found to be 0.999. The precision results were good enough to indicate that the proposed method was precise and reproducible. Preparation of effluent sample was easy and was monitored by UV suitable at λ 293 nm. The assay experiment showed that the content of gatifloxacin estimated was free from the interference of excipients. This demonstrated that the developed HPLC method was simple, fast as evident from short retention time, precise, accurate, sensitive and efficient that could be conveniently adopted for the routine quality control analysis of gatifloxacin from its pharmaceutical dosage forms and bulk drug. The results of forced degradation studies imply that the developed method is stability indicating method.

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Corresponding Author:

Abdul Mannan*,

Email:*syedakulsum@gmail.com*