Trends in Chromatography

Original Communication

Validation of a stability indicating RP-HPLC method for the determination of Gatifloxacin in eye drops

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ABSTRACT

A reversed-phase liquid chromatography (RP-LC) method was validated for the determination of gatifloxacin in eye drops. The LC method was carried out on a Phenomenex LiChrosphere 5 µm RP-18 100 Á 125 x 4.6 mm maintained at room temperature. The mobile phase consisted of water:acetonitrile:triethylamine (80:20:3 v/v/v), pH adjusted to 3.3 using phosphoric acid, run at a flow rate of 1 mL/min and using ultraviolet detection at 293 nm. The chromatographic separation was obtained with a retention time of 3.6 min and was linear in the range of 30–70 µg/mL (r^2 = 0.9991). The specificity and stability indicating the capability of the method was proven through forced degradation studies, which also showed that there was no interference of the excipients. The accuracy was 100.63% with RSD = 1.65. Method validation demonstrates satisfactory results for precision and robustness. The proposed method was applied for the analysis of marketed eye drops, for improving the quality control and to assure the therapeutic efficacy.

KEYWORDS: Gatifloxacin, HPLC, assay, stability-indicating method

INTRODUCTION

Gatifloxacin (±)-1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Figure 1) is the fourth generation of a new class of synthetic antibacterial fluoro-quinolone agents. It is a novel extended spectrum flouroquinolone with an improved Gram positive and anaerobe coverage compared with older agents such as ciprofloxacin. Gatifloxacin has already surpassed the second-generation fluoroquinolones as the antibiotics of choice in cataract surgery because it has a wider spectrum of activity and carries a low risk for resistance developing against it [1].

Most of the analytical techniques for gatifloxacin that are described in the literature are based on the determination of this drug using non-aqueous titration [2], HPTLC [3], UV-spectrophotometric methods [4, 5] spectrofluorimetric determination [6-10] in biological fluids by HPLC with UV detection [11-13] and with other antibiotics [14-16], with fluorimetric detection [1, 17], with mass detection [18].

The proposed HPLC method utilizes economical solvent system, has a better retention time, and very sharp and symmetrical peak shapes. The aim of this study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Gatifloxacin in bulk drug samples and pharmaceutical dosage forms applied in the stability study of the drug.

The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH) [19].

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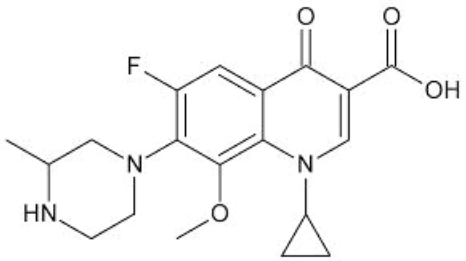

Figure 1. Gatifloxacin.

MATERIALS AND METHODS

Chemical and reagents

Gatifloxacin working standard was provided by Jiangxi Dadi Pharmaceutical Co.LTD (Jiangxi, China) 98.70%, calculated with reference to the dried substance.

An eye-drop formulation was studied. Its composition was Gatifloxacin 500 mg, in a matrix of Disodium EDTA, Sodium chloride and Benzalkonium chloride. Acetonitrile used was HPLC grade, Sintorgan (Buenos Aires, Argentina). Triethylamine was AR grade (Mallinckrodt Baker Inc., Phillipsburg, New Jersey, USA). Distilled water was passed through a 0.45 µm membrane filter.

Equipment

The HPLC system consisted of a dual piston reciprocating Thermo Finnigan pump, a Rheodyne injector and a DAD Dionex Ultimate 3000 with operating software Chromeleon 6.8.

Chromatographic Conditions

The LC method was carried out on a Phenomenex LiChrosphere 5 µm RP-18 100 Å 125 x 4.6 mm maintained at room temperature. The mobile phase consisted of water:acetonitrile:triethylamine (80:20:3 v/v/v) pH adjusted to 3.3 using phosphoric acid, run at a flow rate of 1 mL/min and using ultraviolet detection at 293 nm. The chromatographic separation was obtained with retention time of 3.6 min. The injection volume was 20 µL.

Preparation of standard solution

An accurately weighed quantity of 100 mg of gatifloxacin was dissolved in 100 mL of mobile phase. 5 mL of the resultant solution was pipette out into a 100 mL volumetric flask. The volume was made with mobile phase (Conc 50 µg/mL). The solutions were passed through a 0.45 µm nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota, USA).

Sample preparation

Approximately 5 mL of eye drops were exactly weighed, placed in a 100 mL volumetric flask, taken to volume with mobile phase. 5 mL of the resultant solution was placed in a 25 mL volumetric flask. The volume was made with mobile phase (Conc 50 µg/mL). The solutions were passed through a 0.45 µm nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota , USA).

Method validation

System suitability

Relative standard deviations (RSD) values of the peak area, tailing factor, and retention time were the chromatographic parameters selected for the system suitability test [20].

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. All stress degradation studies were performed at an initial drug concentration of 1 mg/mL, with acid (1 N hydrochloric acid), base (1 N NaOH), water, 100% hydrogen peroxide and refluxing for at least 30 min. The drug was subjected to thermal degradation (either in the solid state or in solution in an open container in an oven at 100 °C for 24 h) and photochemical degradation (a solution was transferred to a container and exposed to daylight for 24 h). After degradation treatment, samples were allowed to cool at room temperature and diluted, if necessary, to the same concentration as that of the standard solution, after being neutralized. After degradation, samples were analyzed using the methodology and the chromatographic conditions described.

Linearity

Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 60 to 150% of assay analyte concentration (i.e. 30, 40, 50, 60 and 70 µg/mL).

Each concentration was repeated three times. The peak area versus concentration data was performed by least-squares linear regression analysis.

Precision

Six injections of standard solution were analyzed to assess system precision. Assay method precision was evaluated by carrying out six independent assays of a commercial formulation of Gatifloxacin against qualified working standard and the percentage of RSD was calculated. The intermediate precision of the method was also verified using different analysts, on different day.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels i.e. 30, 50 and 70 µg/mL of a commercial formulation of gatifloxacin. The percentage recoveries were calculated from the slope and Y-intercept of the calibration curve.

Robustness

To evaluate the robustness of the developed LC method, the chromatographic conditions were deliberately altered and retention time, tailing and efficiency were evaluated. To study the effect, variation of the organic strength of the mobile phase, TEA proportion and pH were altered.

RESULTS AND DISCUSSION

The proposed method was validated as per the guidelines in ICH for its selectivity, linearity, precision, accuracy, and robustness. The described reversed-phase liquid chromatography method was developed to provide a rapid quality control determination of gatifloxacin in eye drops. The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and then the samples were analyzed.

System suitability

System suitability results were calculated according to the USP 35 <621> [20] from typical chromatograms. Instrument precision determined by six successive injections of the Standard preparation provided a relative standard deviation (RSD) below 1.5%. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05}/2f$; where $W_{0.05}$ is the distance from the leading edge to the tailing edge of the peak, measured at 5% of the peak height from the baseline and *f* is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.55.

Stability of the standard solution was studied by injecting the prepared solution at periodic intervals into the chromatographic system during 48 hours stored at room temperature. The solution was at least 99.5 % of their initial concentration under the test conditions.

Selectivity

Gatifloxacin was stable under stress conditions such as acid, alkaline and water hydrolysis, photolytic and thermal conditions. Significant degradation of the drug substance was observed under oxidative hydrolysis, where two degradation peaks at 0.37 and 0.56 RRT, (relative retention time) were detected (Figure 2 and Table 1).

The DAD data shows that purity angle is less than purity threshold and the spectra at peak start, at end and at apex exactly match in the range of 200 nm to 400 nm for all the stressed samples. Hence peak purity test result confirms that the gatifloxacin peak is homogeneous and pure in all the stress samples analyzed. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up 100% of initial value with due consideration to the margin of analytical error. The mass balance of stressed samples was 100.0%. Selectivity was demonstrated showing that gatifloxacin was free of interference from degradation products, and that no interference from the sample excipients was observed at the detection wavelength; thus, the proposed method can be used in a stability assay.

Linearity

Linear calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 60 to 150% of assay analyte concentration and the correlation coefficient obtained was greater than 0.9995. Linearity was checked for the assay method over the same concentration range for two consecutive days. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

The regression line was $y = 89.8866x + 7.3371$ with a correlation coefficient (r^2) of 0.9991. The linearity of the calibration graphs was validated by the high value of the correlation coefficient

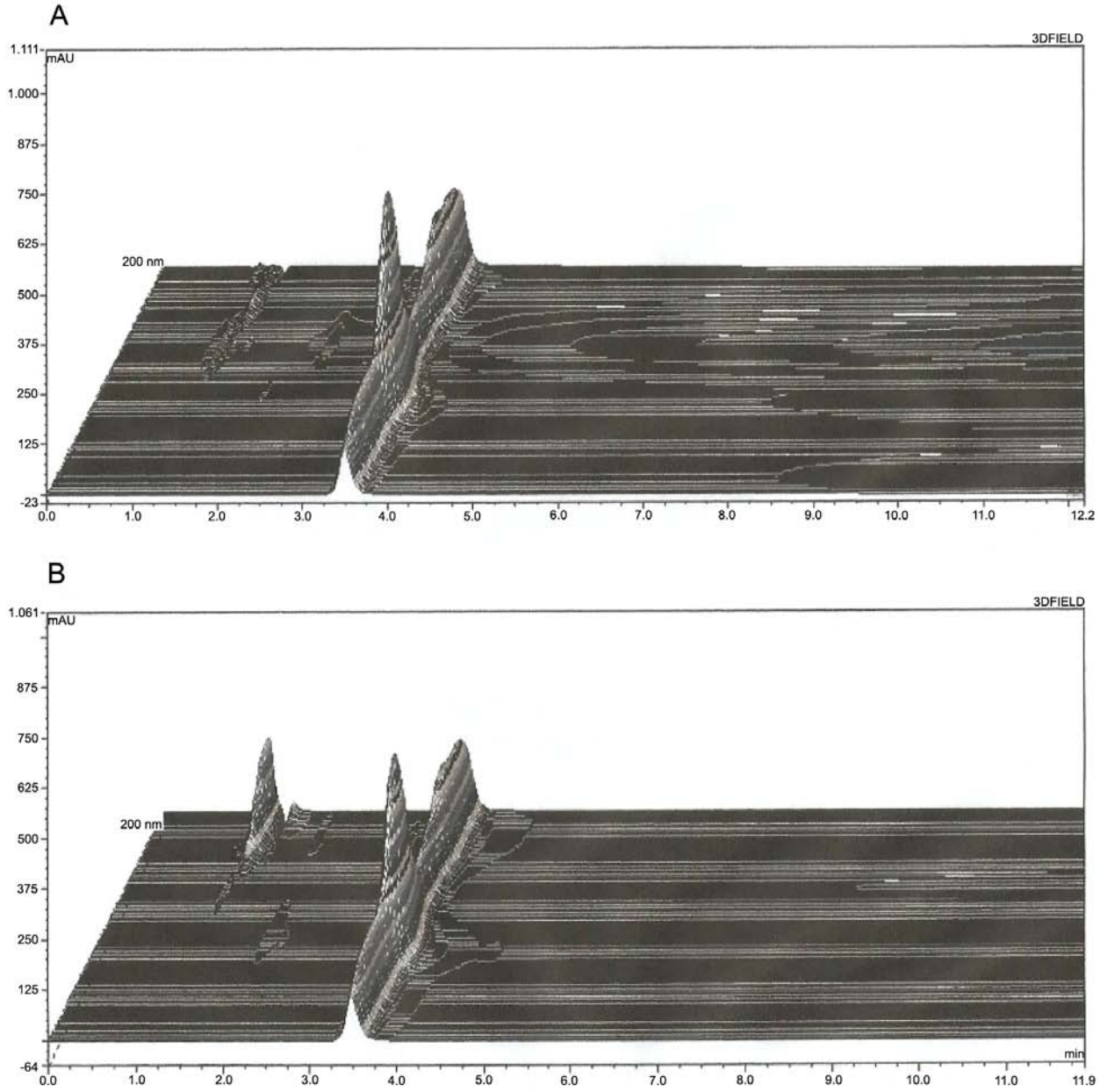

Figure 2. Chromatograms of Gatifloxacin: A - Standard; B - Oxidation (Hydrogen peroxide 100%, reflux, 0.5 h).

and the intercept value that was not statistically ($p = 0.05$) different from zero (Table 2).

Precision

System precision was determined by making six injections of the standard solution. Precision is usually expressed as the RSD of a series of measurements. The RSD of peak area response and retention time showed the satisfactory repeatability of the system (< 1%). The intra-day precision was performed by assaying the samples on two different days and by two different analysts. The results were given both individually and as the average. For each precision assays the results were as follows: mean values 0.5053 g% and 0.5125 g%, RSD 0.93% and 0.48%. Test "*t*" comparing two samples with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 3).

Table 1. Selectivity: degradation conditions of gatifloxacin.

Condition	Time (h)	% of Gatifloxacin	RRT* of degradation products
Acid (1 N HCl, reflux)	0.5	95.0	Non detectable
Base (1 N NaOH, reflux)	0.5	94.7	Non detectable
Hydrogen peroxide 100% (reflux)	0.5	95.4	0.37, 0.56
Water (reflux)	0.5	95.6	Non detectable
Heat dry, 100°C (solution)	24	92.7	Non detectable
Heat dry, 100°C (solid)	24	96.0	Non detectable
Daylight exposure	24	94.8	Non detectable

*RRT, relative retention time.

Table 2. Linearity data of gatifloxacin.

% of nominal value	Injected (µg)	Average peak area response	RSD
60	0.6144	61.6467	0.43
80	0.8192	82.1143	0.20
100	1.0240	100.0663	0.80
120	1.2288	116.7333	0.48
150	1.5360	145.5490	0.18
Slope[a]	89.8866 ± 259.7482		
Intercept[b]	7.3371 ± 283.7421		

[a]Confidence limits of the slope (p = 0.05).
[b]Confidence limits of the intercept (p = 0.05).

Table 3. Precision of the assay method for gatifloxacin.

Analyst 1 Sample N°	g%	RSD (%)	Analyst 2 Sample N°	g%	RSD (%)
1	0.4991	0.56	1	0.5122	0.57
2	0.5063	0.56	2	0.5144	0.57
3	0.5010	0.56	3	0.5125	0.57
4	0.5075	0.56	4	0.5134	0.57
5	0.5072	0.56	5	0.5116	0.57
6	0.5110	0.56	6	0.5108	0.57
Mean	0.5053	0.93	Mean	0.5125	0.48

Accuracy

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percentage difference between the measured mean concentrations and the corresponding nominal concentrations. The accuracy of the proposed method was tested

Table 4. Recovery analysis of gatifloxacin.

% of nominal value	Added amount g%	Found amount g%	Recovery (%)	Average recovery (n = 3)	RSD (%)
	4.0776	4.0693	99.80		
80	4.0680	4.0407	99.33	99.54	0.49
	4.0710	4.0499	99.48		
	5.0905	5.1124	100.43		
100	5.0805	5.0629	99.65	99.69	0.77
	5.0712	5.0200	98.99		
	6.0569	6.1598	101.70		
120	6.0585	6.2875	103.77	102.66	1.01
	6.0795	6.2330	102.52		
Mean (n = 9)				100.63	1.65

Table 5. Robustness.

Mobile Phase	RT Gatifloxacin (Minutes)	Tailing	N
water:acetonitrile:TEA (80:20:3 v/v) pH: 3.3	3.56	1.25	2904
water:acetonitrile:TEA (80:20:3 v/v) pH: 3.0	3.46	1.20	2807
water:acetonitrile:TEA (80:20:3 v/v) pH: 3.5	3.39	1.22	2693
water:acetonitrile:TEA (80:20:3 v/v) pH: 4.0	3.44	1.25	2582
water:acetonitrile:TEA (75:25:3 v/v) pH: 3.3	1.96	1.53	2627
water:acetonitrile:TEA (85:15:3 v/v) pH: 3.3	8.63	1.03	3596

by recovery experiments of one commercial formulation studied (n = 3 for 80%, 100% and 120%). The mean recovery was 100.63% and the RSD was 1.65. The experimental *t* of the recovery percentage whose value was 0.346 was also studied, which is far below the 2.306 established in the tabulated *t* (95% level of probability, 8 d.f) (Table 4).

Robustness

The robustness of the proposed method was found after altering the parameters deliberately: the mobile phase ratio variants ± 5% of water and acetonitrile, pH: -0.3 and +0.2 and +0.7 units. The retention time, tailing and efficiency of the compound were evaluated. Results could be seen in Table 5. There was an important change in the retention time with the change in the mobile phase proportion of water/ acetonitrile which also affects de peak symmetry and efficiency. The variations of pH have not yielded significant changes in retention time and tailing.

CONCLUSIONS

A simple, specific, lineal and precise method was developed for the determination of gatifloxacin in

eye drops. The mobile phase was easy to prepare. The analysis time was found to be less than 4 min. The recovery from formulations was in good agreement and suggested no interference in the estimation. Hence, this method can be easily and conveniently used for the routine quality control of gatifloxacin in pharmaceutical dosage form.

ACKNOWLEDGEMENT

This work was supported by grant 20020100100816 from UBA and PIP Nº: 11420110100380 from CONICET to A. I. Segall.

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