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A validated HPTLC method for estimation of gatifloxacin in tablets

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ABSTRACT

A simple HPTLC method having high accuracy, precision and reproducibility was developed for the routine estimation of Gatifloxacin in the tablets available in market and was validated for various parameters according to ICH guidelines. Gatifloxacin was estimated at 290 nm by densitometry using Silica gel 60 F_{254} as stationary phase and a premix of methylene chloride: methanol: strong ammonia solution and acetonitrile (10:10:5:10) as mobile phase. Method was found linear in a range of 90 monograms to 550 nanograms with a correlation coefficient > 0.99. The regression equation was: AUC = 25.18 × (Amount in nanograms) + 2527 (r^2 = 0.9844).

KEYWORDS

Gatifloxacin;
Validation;
HPTLC.

INTRODUCTION

Gatifloxacin, a fluoroquinolone, [(±) -1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate] has been found effective in acute postoperative endophthalmitis[1], Chromobacterium violaceum infections[2], common respiratory problems[3] and Gram-positive bacterial infections[4] etc. Commercially, it is available as ophthalmic solutions, parenteral solutions and oral tablets. A variety of methods are reported for its estimation using UV spectroscopy[5], microbiological assay[6] or high-performance liquid chromatography[7,8], which are either non-specific, less accurate or expensive. The suggested HPTLC method is specific, accurate, cheaper and less time consuming for routine analysis of Gatifloxacin in bulk and formulations and can be prove of much significance.

EXPERIMENTAL

Instrumentation

Camag HPTLC system (Muttenz, Switzerland) with Linomat 5 sample applicator, TLC scanner 3, HPTLC plate heater III, UV cabinet, 100 µl Hamilton Syringe (Bonaduz, Schweiz), twin trough development chambers (for 10 cm x 10 cm sheets), and winCATS 1.3.4 software was used for the analytical purpose. Merck KGa A coated HPTLC aluminum sheets with Silica gel 60 F_{254} (Darmstadt, Germany) were used as stationary phase. Mettler Toledo balance (Ohio, USA) model XP 205 was used for weighing the chemicals and reagents.

Materials

Gatifloxacin working standard was obtained as a gift sample from Ranbaxy Research Laboratories (Gurgaon, India). Gatifloxacin tablets of three different brands [Lyflox, 400 mg, Lyka Hetero Healthcare Lim-

ited; Gatimac, 400 mg, Macleods Pharmaceuticals Limited; Ragasin, 400 mg, Ranbaxy Laboratories Limited (Stancare)] were collected from market and analyzed by the proposed method. All the other chemicals and reagents were of analytical grade.

Thin layer chromatography (TLC) development

A premix of methylene chloride: methanol: strong ammonia solution and acetonitrile in a ratio of 10:10:5:10 respectively was optimized for TLC plate development. Run distance was kept about 70 mm and 10 ml of the mobile phase was used for single development. The Rf value of Gatifloxacin peak was observed about 0.51. The dosing speed of nitrogen applicator was kept 150 nl sec^{-1} with a pre-dosage volume of 0.2 µl. Samples were applied as bands of 6 mm width with the gaps of 10 mm in between. Developed plates were dried at 40 ^{0}C for 5 min. Detection was done at 290 nm using deuterium lamp in absorption-re-emission mode. The slit dimension of detection was kept 6.00 mm x 0.45 mm, scanning speed 20 mm sec^{-1} and data resolution 100 µm/ step. The various statistical reports were generated according to the standard formulae and parameters were validated as per ICH[9] guidelines.

Specificity and selectivity

Absence of any secondary spot having spectra different from Gatifloxacin, in the typical placebo chromatogram of the tablet preparation, that may interfere with Gatifloxacin peak indicate the specificity of the analytical method (Figure 1 and 2).

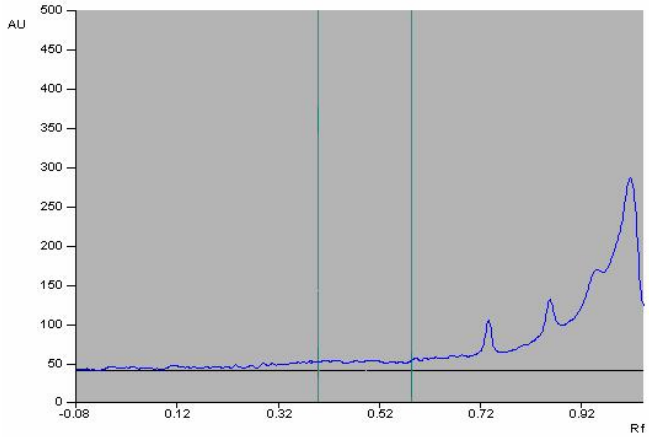

Figure 1 : HPTLC chromatogram of the constituted tablet placebo

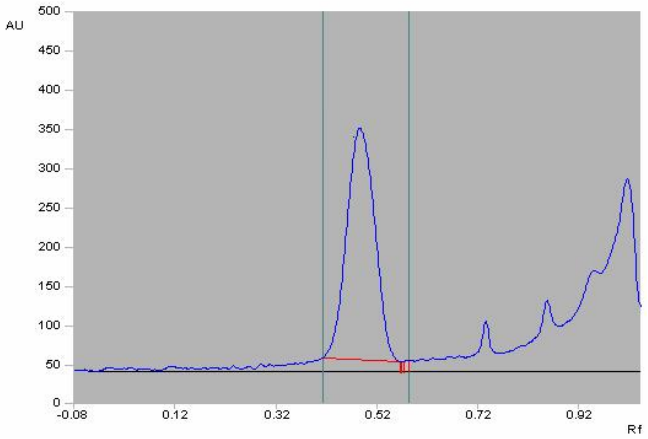

Figure 2 : HPTLC chromatogram of the tablet gatifloxacin (Gatimac)

Calibration standards, linearity and range

Gatifloxacin solution (45 µg ml^{-1}) was prepared in methanol and its 2, 4, 6, 8, 10 and 12 µl volumes were applied on the HPTLC plate as separate spots. The plate was developed, dried and analyzed at 290 nm by densitometry. The calibration data was generated (TABLE 1) and regression analysis (TABLE 2) was performed.

TABLE 1 : Calibration data for linearity

Amount in nanograms per spot	AUC at 290 nm (% RSD) (n=3)
90.5	4055 (0.04)
181.0	7404 (1.60)
271.5	9893 (0.69)
362.0	12061 (0.59)
452.5	13974 (0.15)
543.0	15633 (0.29)

TABLE 2 : Regression analysis

Parameters	Results (n=3)
Equation of the regression line	AUC= 25.18 × (Amount in nanogram) + 2527
Regression coefficient (r^2)	0.9844
Correlation coefficient	0.9921

Precision and formulation analysis

Precision was demonstrated by analyzing the tablet preparations in six replicates. Three different Gatifloxacin tablet samples- Lyflox, Gatimac and Ragasin were prepared by sonicating the tablets in methanol. % Assay calculations were based on the calibration curve. % Relative standard deviation of the %

w/w assay values was reported (TABLE 3).

TABLE 3 : Method precision of the analytical method

Sample/ Spot	Assay (%w/w) Lyflox	Assay (%w/w) Gatimac	Assay (%w/w) Ragasin
Sample/ Spot-1	101.0	101.3	98.2
Sample/ Spot-2	98.4	98.3	99.2
Sample/ Spot-3	99.3	98.6	98.3
Sample/ Spot-4	100.2	100.1	100.1
Sample/ Spot-5	98.3	100.1	98.7
Sample/ Spot-6	98.0	98.3	99.0
Mean	99.2	99.4	98.9
Standard deviation	1.189	1.231	0.689
% RSD	1.20	1.24	0.70

Accuracy

Pre analyzed tablet sample preparations were spiked with Gatifloxacin at three different levels (80 ng, 160 ng 240 ng) and were analyzed in six replicates. Accuracy was reported as % recovery (TABLE 4) based on actual and estimated concentrations.

TABLE 4 : Recovery study

Formulation spiked	Gatimac	Lyflox	Ragasin
Level of spiking (ng)	80	160	240
	% Recovery		
Spot-1	100.1	99.9	98.7
Spot-2	101.6	99.6	101.0
Spot-3	100.5	98.0	99.1
Spot-4	101.7	99.7	97.3
Spot-5	101.7	101.5	98.3
Spot-6	99.9	100.9	101.2
Mean % recovery	100.9	99.9	99.3
SD	0.837	1.230	1.529
% RSD	0.83	1.23	1.54

Ruggedness

Ruggedness of the proposed method was determined by changing the duration of the chamber saturation i.e. 30 ± 10 min. % Assay was reported (TABLE 5).

TABLE 5 : Ruggedness

Formulation	Lyflox	
Condition	Chamber Saturation, 30 +10 min	Chamber Saturation, 30 - 10 min
	% Assay	
Spot-1	101.2	99.7
Spot-2	101.2	99.1
Spot-3	100.2	100.9
Spot-4	99.4	99.7
Spot-5	99.1	98.9
Spot-6	99.5	98.5
Mean % Assay	100.1	99.5
SD	0.895	0.831
% RSD	0.89	0.84

AUC - Area under the curve, SD - Standard deviation, % RSD - Percent relative standard deviation.

RESULTS AND DISCUSSION

The proposed analytical method for assay determination of Gatifloxacin in Tablets was found suitable and applicable to different tablet formulations in market. Method was found linear in a range of ~ 90 to 550 ng with a good correlation of 0.99. A lower % RSD (below 2 %) of % assay values, observed during replicate analysis of different tablets as part of precision, indicate the suitability of the method. A % recovery ranging within 98-102 % demonstrated good accuracy of the analytical method. Additionally, the method was found rugged for chamber saturation time. The proposed method can be extended for assay of Gatifloxacin in other formulations like parenteral preparations or ophthalmic solutions.

ACKNOWLEDGEMENT

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SUPPLEMENTARY INFORMATION AVAILABLE STATEMENT

Supplementary information regarding the entire experimental activities has been provided.

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