Research Article

Development and Validation of New RP-HPLC Method For Simultaneous Estimation of Gatifloxacin and Difluprednate In Pharmaceutical Dosage Forms

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ABSTRACT

A simple, selective, precise and accurate Reverse Phase High Pressure LiquidChromatographic (RP-HPLC) method was developed and validated for the simultaneousestimation ofgatifloxacin and difluprednate in pharmaceutical dosage forms.The method was validated for accuracy, specificity, linearity and robustness asper the ICH guidelines.Binary elution at a flow rate of 1.0 mL per min was employed onEnable C_{18}column at temperature of 25°C. The method was successfully applied to estimategatifloxacin and difluprednate in marketed ophthalmic dosage form since there was nointerference from the excipients.

Keywords: Gatifloxacin, Difluprednate, RP-HPLC, Validation.

INTRODUCTION

Gatifloxacin (GFC) is chemically 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7- (3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxilic acid[1](Fig. 1) has broader spectrum ofAntibacterial activity than the older fluoro quinolones and shows good activity against gram +ve and gram –vemicroorganisms. It inhibits the bacterial enzymes DNA gyrase and topoisomerase-IV. Difluprednate (DFP) is a topical corticosteroid indicated for the treatment of inflammation and pain associated with ocular surgery. It is a butyrate ester of 6(α), 9(α)-difluoro prednisolone acetate[2] (Fig. 2). It is indicated for treatment of endogenous anterior verity. The combination of DFP and GFC in ophthalmic emulsion is used to treat the conjunctivitis. This combination is preferred because difluprednate is a corticosteroid used in inflammatory ocular conditions and along with inflammatory condition, the risk of bacterial infection exists. To prevent this infection gatifloxacin is given in combination.

A detailed literature survey revealed only one bioanalyticalmethod[3] for estimation of difluprednate. Various Spectrophotometricmethods[4-8] and chromatographic methods[9-14] were reported for the estimation of gatifloxacin as individual and with other drug combination. The present communication describes simple, sensitive, rapid, accurate, precise and economical chromatographic method for estimation of both drugs in their combined dosage forms.

MATERIALS AND METHODS

Reagents and chemicals

Raw drugs of GFC and DFP was procured from Dr. Reddy's Laboratories, Hyderabad and marketed dosage form GATILOX-DX procured from Sun Pharma Ltd., Mumbai.Methanol, Acetonitrile, Water, Disodium hydrogen phosphate, Potassium Dihydrogen Phosphate and Water were obtained from Merck Specialities, Mumbai.

Preparation of buffer

Accurately weighed quantities of 1.42 gm of disodium hydrogen phosphate is dissolved in little quantity of HPLC water and after make it up to 1000mL with HPLC water and $P^H$was adjusted to 6.0 with Ortho Phosphoric Acid.

Preparation of mobile phase

A freshly prepared mixture of Methanol and0.01MDisodium hydrogen phosphate in 60:40v/v was used as mobile phase.

HPLC operating conditions

Binary Shimadzu UFLC instrument on aENABLE C_{18} column (250 mm x 4.6 mm,5µ)

was used. The Instrument is equipped with LC-20 AT VP Series binary pump &PDA detector. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using LC-Solutions software. UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Telsonic ultrasonic sonicator. A Shimadzu balance was used for weighing the materials.

PREPARATION OF SOLUTIONS

Preparation of standard stock solution

About 100 mg of DFP and 600 mg of GFC were weighed and transferred into a 100 mL volumetric flask make up with methanol. The solution was sonicated for 15 min.

From the above solution take 10 mL of the solution in to another 100mL volumetric flask make up with methanol. Finally we get 100µg/mL and 600 µg/mL ofDFP and GFC respectively.

Procedure for sample

2mL of ophthalmic solution was transferred into a10 mL volumetric flak and make up with mobile phase and filtered.

Optimization of HPLC method

Various mobile phases were used inorder to find the optimum conditions for the Separation of GFC and DFP from each other. It was foundthat mobile phase containing methanol:0.01M Disodium hydrogen phosphate (P^H 6.0, adjusted with OPA) (60 : 40 v/v), at a flow rate of1.0 mL/min with detection at 242 nm gavesatisfactory results with sharp, well defined andresolved peaks with minimum tailing, ascompared to the other mobile phases. Underthese conditions the retention time was typically 3.01min GFC and 5.9 min DFP shown in Fig. 3.

VALIDATION OF THE METHOD

The ICH[15-19] guidelines were followed for validation ofdeveloped analytical method. The method wasvalidated for the following parameters.

Linearity

From the stock solutions of GFC and DFP 1mL, 2 mL, 3 mL, 4 mL, 5mL, and 6 mL is taken in 6 different 10 mL volumetric flasks and diluted with the mobile phase to give the following Concentrations.

GFC: 60 µg/mL, 120 µg/mL, 180 µg/mL, 240µg/mL, 300 µg/mL,360 µg/mL .

DFP: 10 µg/mL, 20 µg/mL, 30µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL.

Precision

The precision of the method was studied anddetermined by repeatability study and by determining, interday and intraday precision. Repeatability studies were performed by analysisof three different concentrations like 50 %, 100 %and 150 % of the drug for three times on the sameday. Intraday precision studies were carried outby analyzing sample solutions at different timeintervals on the same day. The interday precision studies were determined by injecting the sample solutions on different days.

Accuracy

To confirm the accuracy of the proposed method, recovery studies were carried out by adding aknown amount of the standard solution to a pre-analyzed sample solution. Thesestudies were carried out at 50%, 100 % and 150% levels. The per cent recoveries of GFCand DFPat each level and each replicate were determined.The mean per cent recovery (n=3) of both thedrugs and the relative standard deviation (RSD) were calculated.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, minute variations in the optimized method parameters were done.Forthis purpose, the effect of change in pH (±0.1) of the mobile phase and mobile phase concentrations (±2). Changes occur on the retention time, theoretical plates, areaunder the curve and percent content of GFC and DFP was studied.

RESULTS AND DISCUSSION

The results of validation studies by proposed RP - HPLC are summarized in Table 1.By using the mobile phase of methanol and buffer (60: 40), two drugs, GFCand DFPcould beresolved clearly from each other. The retentiontime for GFC and DFPwas 3.01 and 5.94 min, respectively.

Linearity

The drug response was linear for GFC (r^2 = 0.9999) and for DFP (r^2 = 0.9999) over the concentration range of 60-10 µg/mL (Fig.4 and 5).

Precision

The developed method was found to be preciseas the RSD value for intra-day and interdayprecision studies was < 2 %, which is in the limitas per the recommendations of ICH guidelines.

Analysis of marketed ophthalmic dosage form

Experimental results for the amount ofGFC and DFPin marketed ophthalmic dosage form were in good agreementwith their label claims. This suggested that therewas no interference from any of the excipients,which are normally present in marketed ophthalmic dosage form. The replicate analysis (n=6) of GFC and DFP by theproposed method showed that the content ofGFC and DFPinthe ophthalmic dosage formwas 99.8 % and 100.2 %, respectively. The results are summarized in Table 2.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of GFC and DFPfrom theirmixture in the marketed ophthalmic dosage form is simple, rapid, selective, accurate, precise, linear and robust. Hence, it canbe adopted efficiently and easily for routine quality control analysis with accuracyand reproducibility of the results.

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Table 1: Summary of Validation Results

Parameters	GFC	DFP
Linearity	R^2 =0.9999	R^2 =0.9999
Range	60-360(µg/mL)	10-60 (µg/mL)
Accuracy	% recovery = 99.83	% recovery = 100.7
System precision	%RSD = 1.73	%RSD = 0.69
Method precision	% assay = 1.67	% assay = 0.65
LOD	6.5	1.1
LOQ	21.33	3.5
Robustness	Robust	Robust
% assay	99-101%	99-101%

Table 2: Summary of analysis of marketed ophthalmic dosage form

Brand Name	Drug	Labeled amount	Amount found	% Assay
GATILOX-DX	Gatifloxacin	3mg	2.98	99.3
	Difluprednate	0.5mg	0.49	99.2

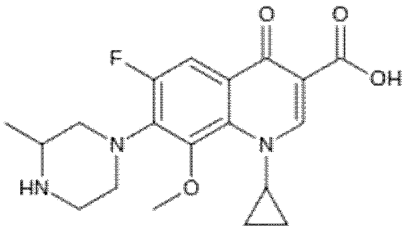

Fig. 1: Structure of gatifloxacin

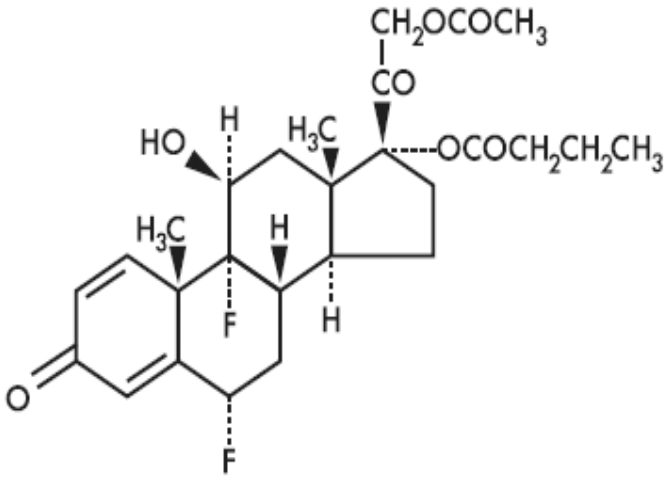

Fig. 2: Structure of difluprednate

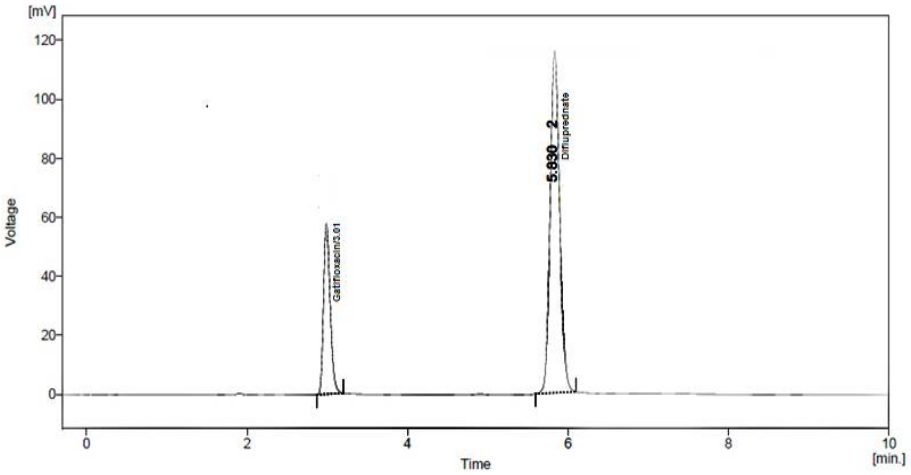

Fig. 3: Chromatogram of a mixture of gatifloxacin and difluprednate

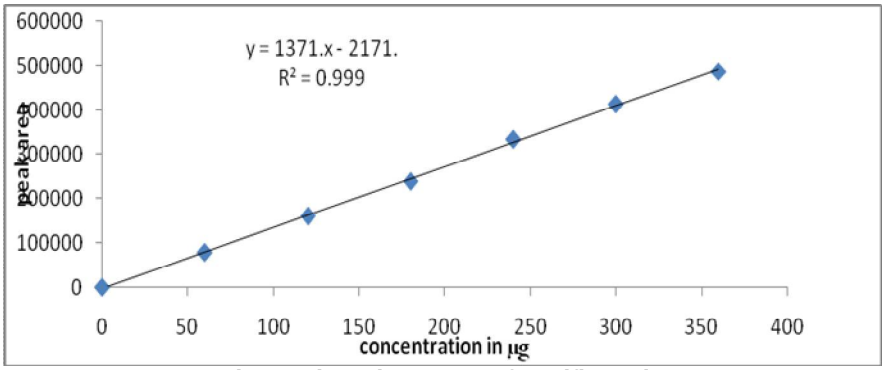

Fig. 4: Linearity Curve of Gatifloxacin

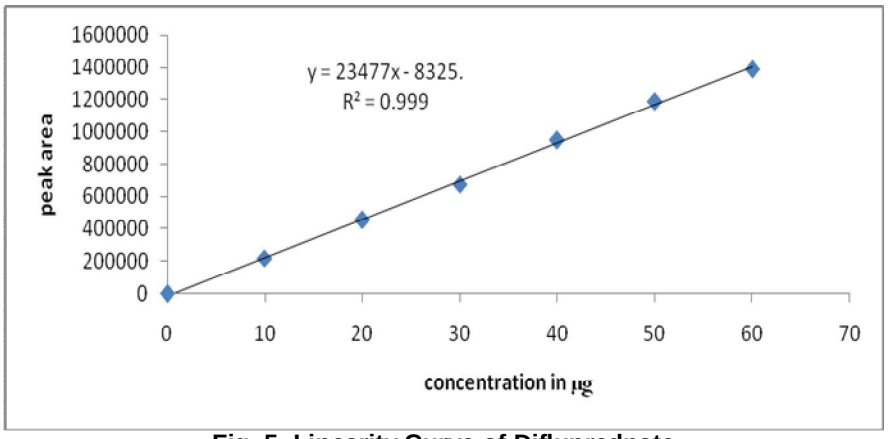

Fig. 5: Linearity Curve of Difluprednate

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