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RESEARCH ARTICLE

VALIDATED RP- HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DIFLUPREDNATE AND GATIFLOXACIN IN OPTHALMIC EMULSION

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Abstract

A simple, precise, accurate, selective and economical RP-HPLC method has been developed and validated for the simultaneous estimation of difluprednate and gatifloxacin in eye drops formulation. The separation was carried out using a mobile phase consisting of Water and Acetonitrile with pH 2.0 adjusted with orthophosphoric acid in the ratio of 25:75 % v/v. The column used was Shim pack XR ODS II (150 mm x 3 mm id, 5 µm) with flow rate of 1 ml / min using UV detection at 263 nm. The described method was linear over a concentration range of 1-6 µg/ml and 2-14 µg/ml for the assay of difluprednate and gatifloxacin respectively. The retention times of difluprednate and gatifloxacin were found to be 5.23 ± 0.0374 and 2.55 ± 0.0296 min respectively. Method was validated statistically and recovery studies were carried out. The proposed method has been applied successfully to the analysis of cited drugs either in pure form or in pharmaceutical formulations with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.

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INTRODUCTION

Difluprednate (DFBA) is a topical corticosteroid indicated for the treatment of infammation and pain associated with ocular surgery. It is a butyrate ester of 6(α), 9(α)-difluoro prednisolone acetate. Difluprednate is abbreviated DFBA, or difluoroprednisolone butyrate acetate. It is indicated for treatment of endogenous anterior verity. Gatifloxacin (GATI) chemically is 1-cyclopropyl-6-fluoro- 8- methoxy-7-(3-methylpiperazin-1-yl) - 4-oxo-quinoline-3-carboxylic acid. It is an antibiotic belongs to Fluoroquinolone family. It is an 8-methoxy Fluoroquinolone with invitro activity against a wide range of gram-negative and gram-positive micro organisms. It inhibits the bacterial enzymes DNA gyrase and topoisomerase-IV. It is available for oral and parenteral administration. [1]

The combination of DFBA and GATI in ophthalmic emulsion form is use to treat the conjunctivitis. This combination is preferred because DFBA is a corticosteroid used in inflammatory ocular conditions and along with inflammatory condition, the risk of bacterial infection exists. To prevent this infection GATI is given in combination.

A detailed literature survey revealed only one bioanalytical method[2] for estimation of DFBA. Various spectrophotometric method[3-8] , HPLC[9-11], RP-HPLC[12-14], method for GATI as individual and with other drug combination are also available. The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of GATI and DFBA in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of GATI and DFBA in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for estimation of both drugs in their combined dosage forms.

This paper describes simple, accurate, precise and economical method for simultaneous determination of DFBA and GATI in ophthalmic emulsion. The chemical structure of DFBA and GATI is shown in the (Fig 1 and 2).

MATERIALS AND METHODS

Instruments
RP-HPLC instrument (Shimadzu, LC-20 AD) equipped with a UV-Visible detector, a Shim pack XR ODS II 150 mm x 3 mm id, 5 µm column was used. Chromatograms were automatically obtained by LC-solution system software.

Reagents and Chemicals
DFBA and GATI were obtained as a gift sample from Sun Pharmaceuticals Industries Ltd, Vadodara. Methanol, Acetonitrile, Orthophosphoric acid and distilled water was used in the study. The commercial fixed dose combination product containing 0.5 mg DFBA and 3 mg GATI was procured from the local market. All other chemicals and reagents used were of HPLC grade.

Liquid chromatographic conditions
Chromatographic separations were obtained by gradient elution mode which was performed using a mobile phase containing Water and Acetonitrile (pH adjusted to 2.0 using orthophosphoric acid) in the ratio of 25:75 % (v/v) at a flow rate of 1ml/min through Shim pack XR ODS II (150 mm x 3 mm id, 5 µm). The selective detection of the column effluent was monitored at a wavelength of 263 nm. Injection volume was 20µl.

Preparation of standard stock solutions
Standard stock solutions containing DFBA and GATI were prepared individually by dissolving 10 mg of DFBA and 10 mg of GATI in 20 ml of methanol. It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 100 ml with HPLC grade water to get stock solutions containing 100µg/ ml each of DFBA and GATI in two different 100 ml volumetric flasks.

Preparation of sample solution
1ml of sample stock solution was taken from the formulation and transferred to 10 ml volumetric flask and diluted up to 10 ml with water, which is equivalent to 10 µg/ml DFBA and 60 µg/ml GATI. From this stock, 2 ml of solution was taken and diluted up to 10 ml with water which contain 2 µg/ml DFBA and 12 µg/ml GATI. The resulting solution was filtered through 0.45µ membrane filter.

VALIDATION OF THE PROPOSED METHOD
The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [15].

Calibration curve (Linearity)
Appropriate aliquots of DFBA working standard solution were taken in different 10 ml volumetric flasks. Appropriate aliquots of GATI working standard solution were added to the same flasks. The volumes were made up to the mark with mobile phase to obtain final concentrations of 1, 2, 3, 4, 5, and 6 µg/ml of DFBA and 2, 4, 6, 8, 10, 12 and 14 µg/ml GATI, respectively. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed and regression equations were computed for DFBA and GATI.

Precision (Repeatability)
The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for DFBA and GATI (4 µg/ml for DFBA and 8 µg/ml for GATI) without changing the parameter of the proposed chromatographic method.

Intermediate Precision
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of

standard solutions of DFBA (3, 4, and 5μg/ml) and GATI (4, 6 and 8 μg/ml). The result was reported in terms of relative standard deviation (% RSD).

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

The regression analysis data and validation parameters for the methods are shown in (Table 1). The calibration curve for DFBA and GATI is shown in (Figure 3 and 4). The method was found to be precise and accurate which was evident from its low %RSD values (Table 2 and 3). The results of the assay are shown in the (Table 4). Results of robustness study are shown in (Table 5). The results for system suitability are shown in (Table 6). Chromatogram of Standard DFBA and GATI are shown in the (Figure 6 and 7).

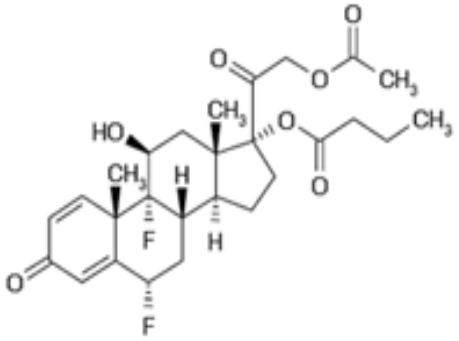

Figure 1. Chemical structure of DFBA

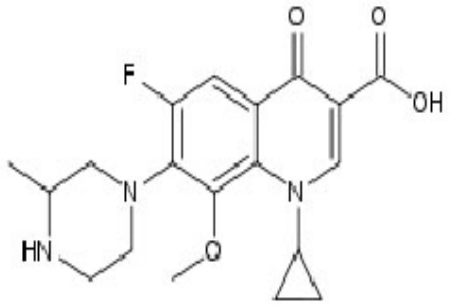

Figure 2. Chemical structure of GATI

Table 1. Regression analysis data and summary of validation parameters for the proposed method

Parameters		RP-HPLC method	
		DFBA	GATI
Concentration range (µg/ml)		1-6	2-14
Slope		13644	33076
Intercept		1066	10633
Correlation coefficient		0.998	0.996
LOD (µg/ml)		0.204	0.269
LOQ (µg/ml)		0.618	0.815
Accuracy ± S.D (n=3)	80%	100.14 ± 0.0109	100.27 ± 0.01732
	100%	99.83 ± 0.0288	99.66 ± 0.1212
	120%	100.14 ± 0.0288	99.73 ± 0.1443
Repeatability (% RSD, n=6)		1.59	1.02
Intraday precision (n=3)		0.13-1.09	0.21-1.73
Interday precision (n=3)		0.13-1.24	0.16-1.31
Assay ± S.D		100.66 ± 0.0288	100.24 ± 0.1212

Table 2. Statistical analysis for precision of proposed method

Drug	Conc. of drug (µg/ml)	%RSD (n=3)	
		Intraday	Interday
DFBA	3	0.30	0.87
	4	1.09	1.24
	5	0.13	0.13
GATI	4	0.21	0.16
	6	0.47	0.55
	8	1.73	1.31

Table 3. Statistical analysis for accuracy of proposed method

DRUGS	Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Total amount of drug (µg/ml)	%Recovery (n = 3)	%RSD
DFBA	80%	1	0.8	1.8	100.14	0.60
	100%		1	2	99.83	1.44
	120%		1.2	2.2	100.14	1.31
GATI	80%	6	4.8	10.8	100.27	0.15
	100%		6	12	99.66	1.01
	120%		7.2	13.2	99.73	1.10

Table 4. Analysis of DFBA and GATI in marketed formulation

Formulation (eye drops)	Labeled amount (mg/ml)		Amount found (mg/ml)		% Label claim ± S.D Assay	
	DFBA	GATI	DFBA	GATI	DFBA	GATI
	0.5	3	0.502	3.007	100.66 ± 0.0288	100.24 ± 0.1212

Table 5. Results of Robustness study

Sr. No.	Parameters	Variation	Assay % of DFBA	Assay % of GATI
1	Flow rate (±0.2 ml/min)	a) 0.8 ml/min	100.33±1.25	100.24±1.01
		b) 1.0 ml/min	99.5±0.86	101.08±0.44
		c) 1.2 ml/min	100.83±0.57	100.50±1.29
2	Wavelength (±2 nm)	a) 261 nm	100.27±0.1.15	99.92±1.45
		b) 263 nm	101.16±0.57	100.76±1.45
		c) 265 nm	100.16±1.15	101.34±0.44
3	Mobile phase composition %v/v (± 2)	a) 73:27	100.03±1.32	99.77±0.91
		b) 75:25	99.66±0.76	100.03±1.35
		c) 77:23	100.83±1.15	100.56±1.19

Table 6. Results of System suitability parameters

Parameters	Data obtained	
	DFBA	GATI
Retention Time	5.23 ± 0.0374	2.55 ± 0.0296
Theoretical plates per column	8700.64 ± 37.97	5906.76 ± 39.7193
Symmetry factor/Tailing factor	1.32 ± 0.037	1.26 ± 0.037
Capacity factor	1.019	1.032
Separation factor	1.817	
Resolution	5.78	

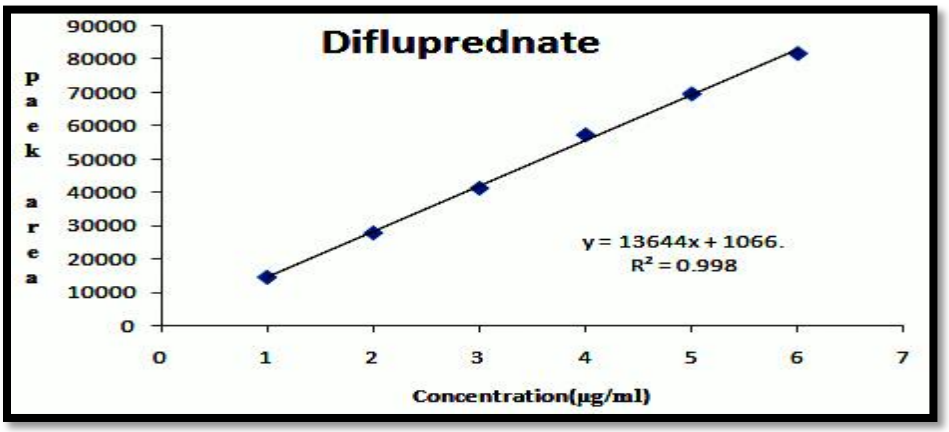

Fig 3. Calibration curve of DFBA

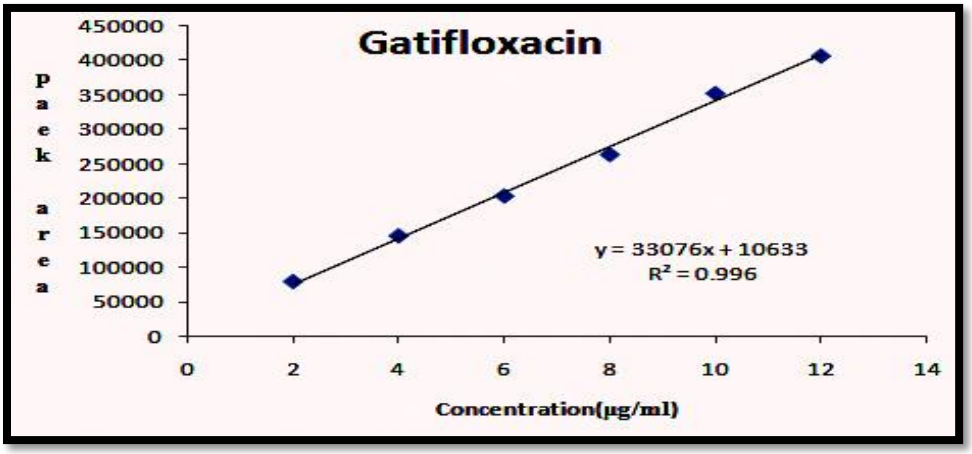

Fig 4. Calibration curve of GATI

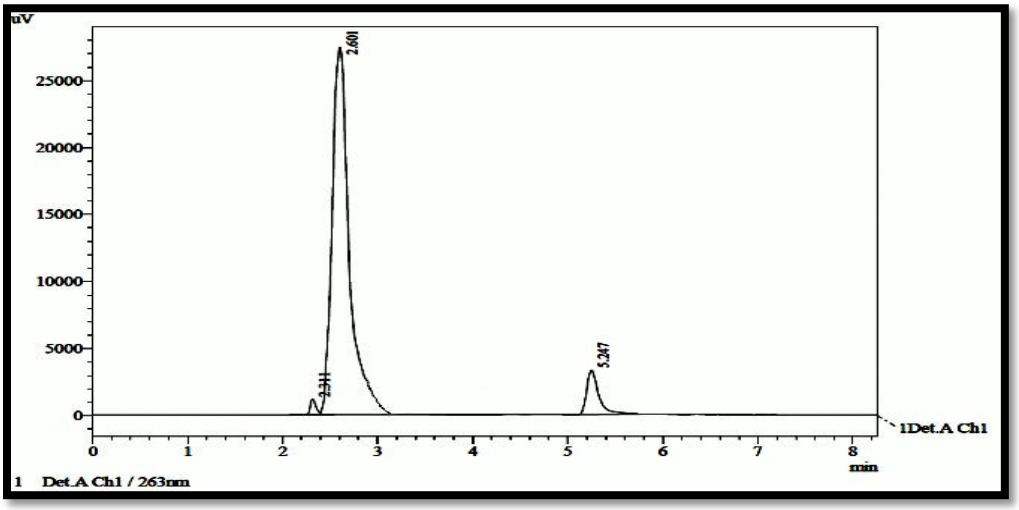

Fig 5.Chromatogram of GATI and DFBA

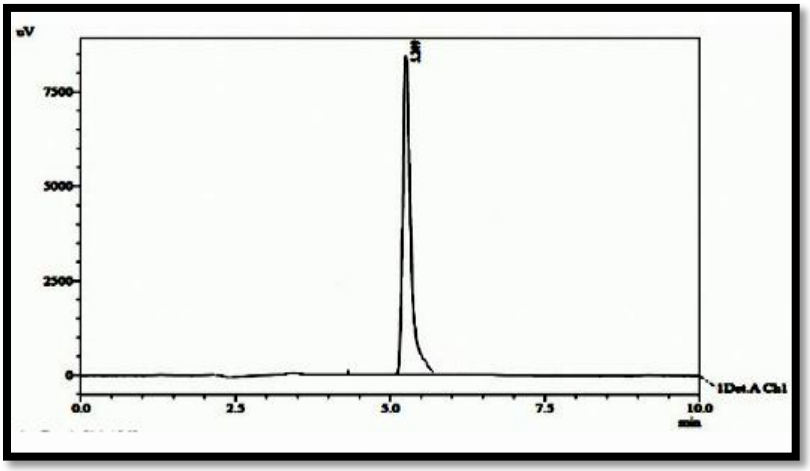

Fig 6. Standard chromatogram of DFBA

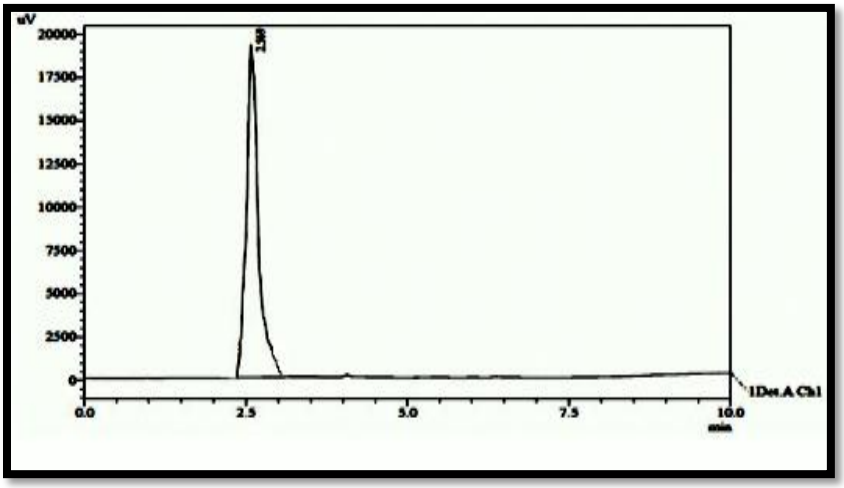

Fig 7. Standard chromatogram of GATI

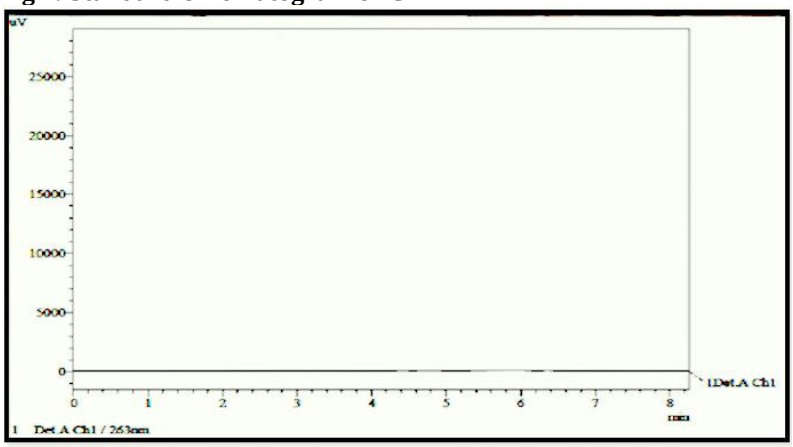

Fig 8. Chromatogram of baseline

CONCLUSION

In this proposed method, the linearity is observed in the concentration range of 1-6 µg/ml for DFBA and 2-14 µg/ml for GATI with co-efficient of correlation, (R^2) = 0.9985 and (R^2) = 0.9961 for DFBA and GATI, respectively at 263 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the DFBA and GATI in combined dosage form without any interference of excipients.

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