International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 3 Issue 4‖ April 2014 ‖ PP.01-10

Development and Validation Of Analytical Methods For Simultaneous Estimation of Difluprednate And Gatifloxacin In Ophthalmic Emulsion By Uv- Visible Spectroscopy

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ABSTRACT :*Two simple, sensitive, precise, accurate and economical UV-Spectrophotometric method developed for the quantitative estimation of difluprednate and gatifloxacin in bulk and combined ophthalmic emulsion. The first method involved determination of difluprednate and gatifloxacin using the Q-absorption ratio method, which involves the formation of Q-absorbance equation at 236 nm (isoabsorptive point) and at 241 nm, which is λ-max of difluprednate. The linearity was obtained in the concentration range of 1-30 ug/ml for both the drugs. The second method involved determination of these two drugs using the first-derivative spectrophotometric techniques were made at 322.60 nm (ZCP of difluprednate) for gatifloxacin and 263.20 nm (ZCP of gatifloxacin) for difluprednate. The linearity was obtained in the concentration range of 5-35 µg/ml for difluprednate and 10-70µg/ml for gatifloxacin. These methods were successively applied to pharmaceutical formulations because no interferences from the ophthalmic emulsion excipients were found. The suitability of these methods for the quantitative determination of the compounds was proved by validation.*

KEY WORDS: *Difluprednate,gatifloxacin, Q-absorption ratio method, first derivative spectrophotometric techniques, Validation.*

I. 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 difluprednate and gatifloxacin in ophthalmic emulsion is use 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 bioanalytical method[2] for estimation of difluprednate. Various spectrophotometric method[3-7] , HPLC[8-10], RP-HPLC[11-13], method for gatifloxacin 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 gatifloxacin and difluprednate in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of gatifloxacin and difluprednate 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. The chemical structure of difluprednate and gatifloxacin is shown in the (Fig 1 and 2).

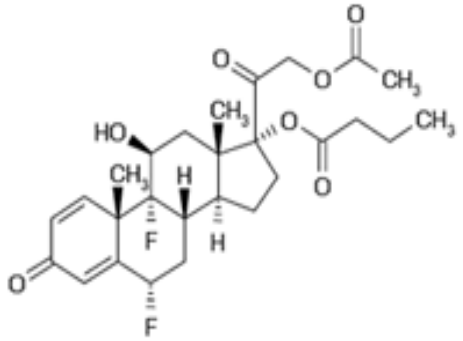

Fig.1 Chemical structure of difluprednate

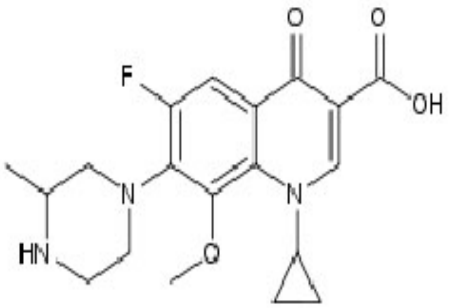

Fig.2 Chemical structure of gatifloxacin

II. MATERIALS AND METHODS

2.1 Reagents & Instruments

A UV-VIS spectrophotometer Shimadzu UV-1800, Software UV-probe 2.33 and Shimadzu ATX 224 electronic balance was used for the experimental purpose. Methanol and double distilled water was used in the study. Difluprednate and gatifloxacin were obtained as a gift sample from Sun Pharmaceuticals Industries Ltd, Vadodara. All the other reagents used were of analytical grade.

Preparation of Standard Drug Solution:

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 water to get stock solutions containing 100µg/ mL each of DFBA and GATI in two different 100 ml volumetric flasks.

2.2 Determination of Absorption Maxima:

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg/ml of DFBA and GATI were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. DFBA and GATI s howed absorbance maxima at 241nm and 286 nm respectively. The overlain spectra showed λ-max of both drugs and also isoabsorptive points at 236 nm (Fig. 3).

III. METHODOLOGY

3.1 Q-Absorption Ratio Method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that difluprednate and gatifloxacin show an isoabsorptive point at 236 nm. The second wavelength used is 241 nm, which is the λ-max of difluprednate. Six working standard solutions having concentration range of 1-30 ug/ml for both the drugs were prepared in distilled water and the absorbances at 236 nm (isoabsorptive point) and 241 nm (λ-max of DFBA) were measured and absorptivity coefficients were

calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

$Cx = \{(Q_M - Qy)/ (Qx - Qy)\} * (A_1/ax_1)$
$Cy = A/ ax_1 - Cx$
Where,

$$Q_M = \frac{\text{Absorbance of sample at 241nm}}{\text{Absorbance of sample at 236 nm}}$$

$$Q_X = \frac{\text{Absorptivity of DFBA at 241 nm}}{\text{Absorptivity of DFBA at 236 nm}}$$

$$Q_Y = \frac{\text{Absorptivity of GATI at 241 nm}}{\text{Absorptivity of GATI at 236nm}}$$

A = Absorbance of sample at iso-absorptive point
ax_1 = Absorptivity of DFBA at iso-absorptive point.

3.1.2 First derivative spectrophotometric method

The standard solutions of DFBA (10µg/ml) and GATI (5µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. The two spectra were overlain and it appeared that difluprednate showed zero crossing at 322.60 nm, while gatifloxacin showed zero crossing at 263.20 nm. At the zero crossing point (ZCP) of difluprednate (322.60 nm), gatifloxacin showed an absorbance, whereas at the ZCP of gatifloxacin (263.20 nm), difluprednate showed an absorbance. Hence 263.20 and 322.60 nm was selected as analytical wavelengths for determination of difluprednate and gatifloxacin, respectively. The linearity was obtained in the concentration range of 5-35 µg/ml for difluprednate and 10-70µg/ml for gatifloxacin and the absorbances for both were measured at respective Zero crossing point.

3.2 Assay of Difluprednate and Gatifloxacin from Formulation

1 ml emulsion was taken from formulation having label claim 0.5 mg/ml DFBA and 3 mg/ml GATI and transferred to 100 ml volumetric flask and dissolved in methanol and diluted upto 100 ml with distilled water, which is equivalent to 5 µg/ml of DFBA and 30 µg/ml of GATI. The mixture was allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter to get clear solution. For Q-absorption ratio method the absorbance of resulting solutions were measured at 241 nm, 236nm (iso-absorptive point). For First derivative method the absorbance was measured at 263.20 nm (ZCP of DFBA) and 322.60 nm (ZCP of GATI).

IV. METHOD VALIDATION

The described methods have been validated for the assay of both the major components of bulk drug using following ICH parameters. [14]

4.1 Linearity

Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 1-30 µg/ml for difluprednate and gatifloxacin in Q-absorption ratio method and for First derivative method, the calibration curves were plotted over a concentration range of 5-35 µg/ml for difluprednate and 10-70µg/ml for gatifloxacin and linear regression analysis was carried out.

4.2 Precision

4.2.1 Intraday

For Q-absorption ratio method, mixed solution containing 10, 15 and 20 µg/ml of both drugs and for First derivative method 15, 20, 25 µg/ml for DFBA and 30, 40 and 50 µg/ml for GATI was analyzed three times on same day and %R.S.D was calculated.

4.2.2 Interday

For Q-absorption ratio method, mixed solution containing 10, 15 and 20 µg/ml of both drugs and for First derivative method 15, 20, 25 µg/ml for DFBA and 30, 40 and 50 µg/ml for GATI was analyzed on three different days and %R.S.D was calculated.

4.3 Accuracy

4.3.1 For Q-absorption ratio method

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of three different levels. Accuracy was determined by calculating recovery of DFBA and GATI by the standard addition method. From working sample solution of test, 1 ml of solution were taken and increasing aliquots of combined working standard solution (0.16, 0.2 and 0.44 ml from 100 µg/ml of DFBA and 0.96, 1.2, 1.14 ml of GATI) were added and diluted to 10 ml with distilled water.

These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for DFBA and GATI. Results of recovery studies were presented in Table III.

4.3.2 For First derivative spectroscopic method

From working sample solution of test, 1 ml of solution was taken and increasing aliquots of combined working standard solution (0.4, 0.5 and 0.6 ml from 100 µg/ml of DFBA and 2.4, 3.0, 3.6 ml of GATI from 100µg/ml) were added and diluted to 10 ml with distilled water.

These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for DFBA and GATI. Results of recovery studies were presented in Table VII.

4.4 Limit of detection and limit of quantitation

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined on the basis of response and slope of the regression equation.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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

V. RESULTS AND DISCUSSION

5.1 For Q-absorption ratio method

Results of optical and regression analysis are shown in Table 1. The accuracy of the method was confirmed by recovery studies from the formulation at three different levels of standard additions. The calibration curve is shown in (Fig 6 and 7). The method was found to be accurate and precise which was evident from its low % RSD values (Table 1 and 2). Linearity spectra of difluprednate and gatifloxacin are shown in (Fig 4 and 5). Q-absorption spectra showing iso absorptive point is shown in (Fig 3).

5.2 For First order derivative spectroscopic method

Results of optical and regression analysis are shown in Table 5. The accuracy of the method was confirmed by recovery studies from the formulation at three different levels of standard additions. The calibration curve is shown in (Fig 10 and 11). The method was found to be accurate and precise which was evident from its low % RSD values (Table 5 and 6).

TABLE 1.Regression analysis of Q-absorption ratio method

PARAMETERS		DFBA	GATI
Concentration range (µg/ml)		1-30 µg/ml	1-30 µg/ml
Molar Absorptivity (L/mol/cm)		1.6×10^5	1×10^5
Sandell's Sensitivity ($\mu g/cm^2$ /0.001absorbance unit)		0.00317	0.00375
Slope		0.0333	0.0283
Intercept		0.0057	0.0002
Correlation coefficient		0.9992	0.9988
Accuracy (recovery, n = 3)	80%	99.51 ± 0.03	100.23 ± 0.05
	100%	99.52 ± 0.06	100.13 ± 0.03
	120%	100.58 ± 0.02	99.82 ± 0.08
Precision (RSD), %			
Repeatability (RSD, n = 6), %		0.165	0.197

Intraday (n = 3)	0.11 – 0.79	0.31 – 0.43
Interday (n = 3)	0.23 – 0.52	0.26 – 0.61
LOD (mcg/ml)	0.05	0.06
LOQ (mcg/ml)	0.173	0.20

TABLE 2.Results of precision study for Q-absorption ratio method (Intra-day and inter-day)

Drug	**Conc. of drug (µg/ml)**	**%RSD (n=3)**	
		Intraday	**Interday**
Difluprednate	10	0.353	0.529
	15	0.115	0.231
	20	0.797	0.352
Gatifloxacin	10	0.433	0.618
	15	0.423	0.269
	20	0.313	0.427

TABLE 3. Recovery study of difluprednate and gatifloxacin by Q-absorption ratio method

DRUGS	**Recovery level**	**Amount present (µg/ml)**	**Amount spiked(µg/ml)**	**Total amount of drug(µg/ml)**	**%recovery (n = 3)**	**%RSD**
DFBA	80%	2	1.6	3.6	99.51	0.839
	100%		2	4	99.54	1.549
	120%		4.4	4.4	100.58	0.603
GATI	80%	12	9.6	21.6	100.23	0.207
	100%		12	24	100.13	0.145
	120%		14.4	26.4	99.82	0.306

TABLE 4.Analysis of DFBA and GATI in marketed formulation by Q-absorption ratio method

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	0.48	2.99	100.08 ± 0.152	99.66 ± 0.1

TABLE 5. Optical and Regression Analysis Data and Validation Parameter of First Order Derivative Method

PARAMETERS		**DFBA**	**GATI**
Concentration range(mcg/ml)		5-35 µg/ml	10-70 µg/ml
Molar Absorptivity (L/mol/cm)		0.4576×10^6	0.2252×10^6
Sandell's Sensitivity (µg/cm^2/0.001 absorbance unit)		0.0011	0.0016
Slope		0.0009	0.0006
Intercept		0.0003	0.0010
Correlation coefficient		0.9990	0.9986
Accuracy (recovery, n = 3)	80%	100.38 ± 0.20	99.89 ± 0.25
	100%	99.6 ± 0.20	100.9 ± 0.95
	120%	100.66 ± 0.11	99.32 ± 0.50
Precision (RSD), %			
Repeatability (RSD, n = 6), %		1.75	0.87
Intraday (n = 3)		1.46 – 1.65	0.91 – 1.43
Interday (n = 3)		0.83 – 1.00	0.00 – 1.14
LOD (mcg/ml)		0.634	0.632
LOQ (mcg/ml)		1.922	1.916

TABLE 6.Results of precision study by First Order Derivative Method (Intra-day and inter-day)

DRUG	Conc. of drug (µg/ml)	%RSD (n=3)	
		Intraday	Interday
Difluprednate	15	1.65	0.83
	20	1.46	0.93
	25	1.49	1.00
Gatifloxacin	30	1.14	1.14
	40	1.43	1.14
	50	0.91	0.91

TABLE 7. Recovery study of synthetic mixtures of difluprednate and gatifloxacin by First Order Derivative Method

DRUGS	Recovery level	Amount present (µg/ml)	Amount spiked(µg/ml)	Total amount of drug(µg/ml)	%recovery (n = 3)	%RSD
DFBA	80%	5	4	9	100.38	1.84%
	100%		5	10	99.60	1.74%
	120%		6	11	100.66	1.14%
GATI	80%	30	24	54	99.89	0.46%
	100%		30	60	100.90	1.54%
	120%		36	66	99.32	0.77%

TABLE 8. Analysis of DFBA and GATI in marketed formulation by First Order Derivative Method

Formulation (eye drops)	Labeled amount (mg/ml)		Amount found (mg/ml)		% Label claim ± S.D Assay (n = 3)	
	DFBA	GATI	DFBA	GATI	DFBA	GATI
	0.5	3	0.53	2.97	100.3 ± 0.64	99.07 ± 0.96

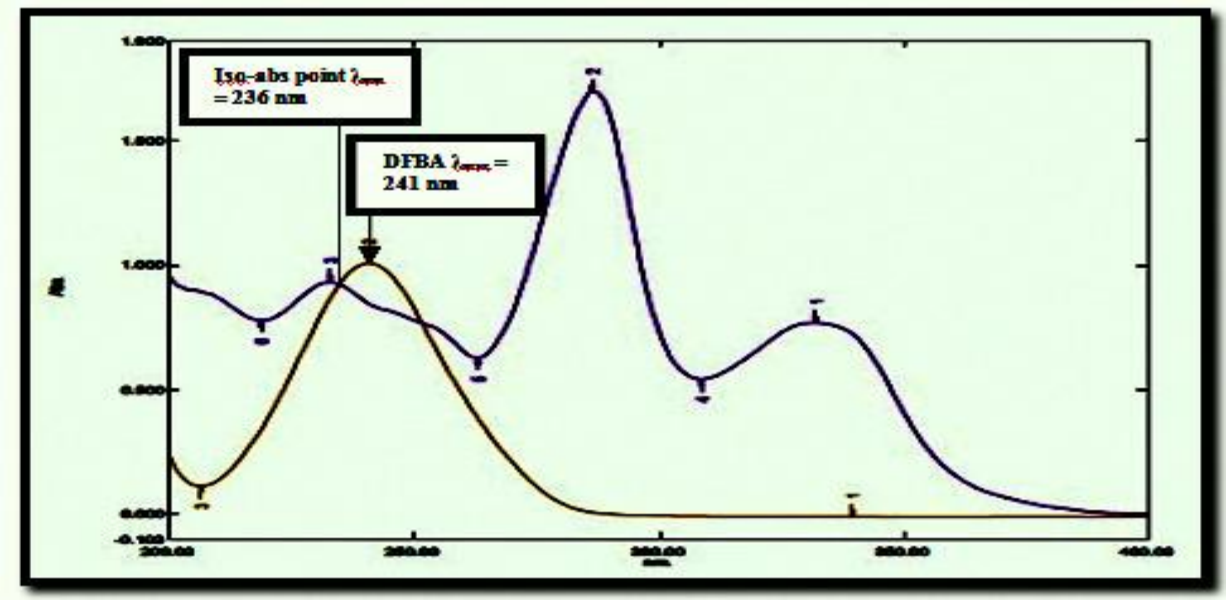

Fig.3 Q-Absorption spectra at iso absorptive point 236 nm

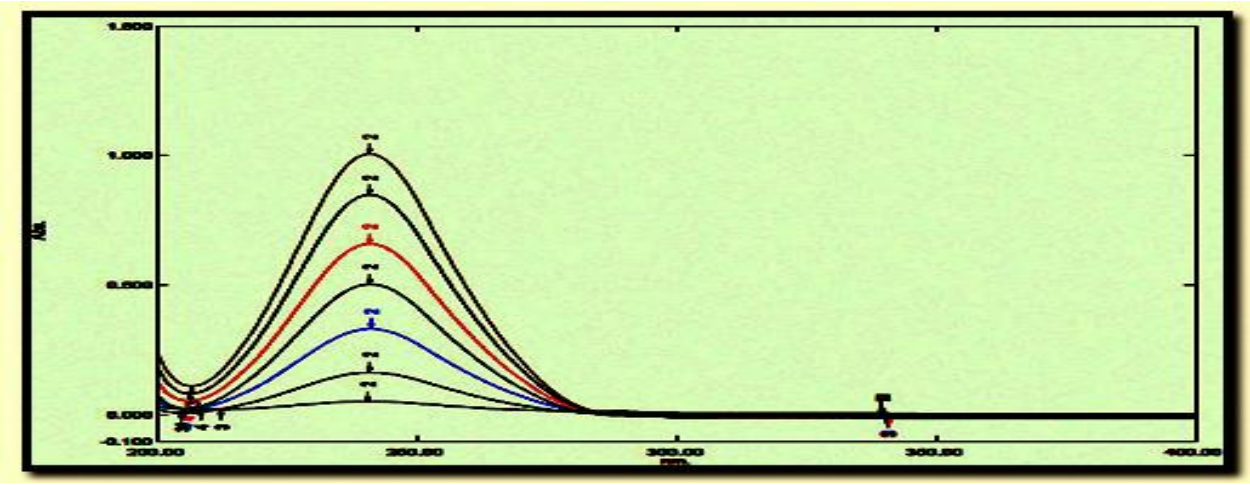

Fig.4 Overlain spectra of difluprednate at 241 nm

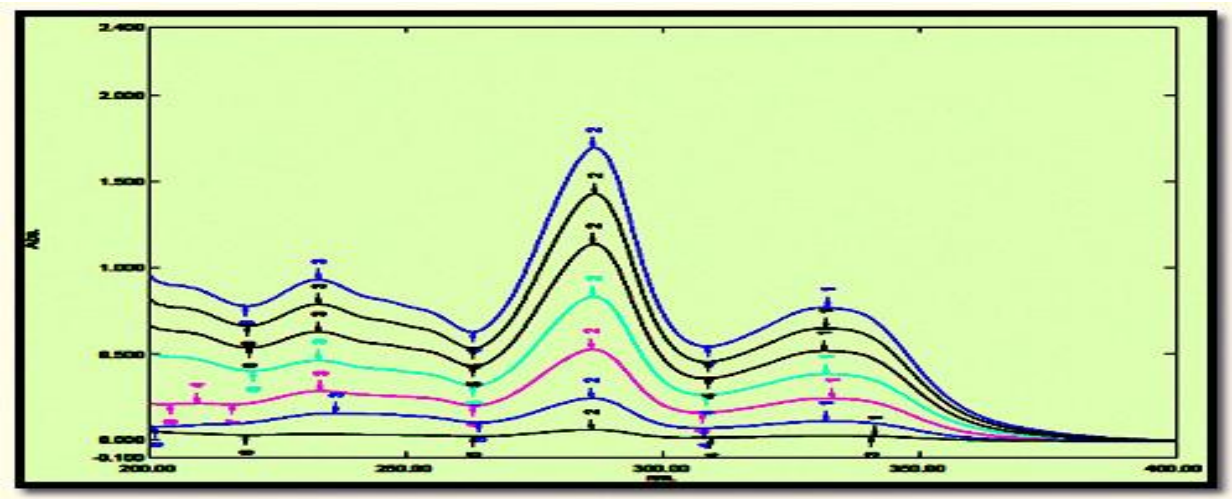

Fig.5 Overlain spectra of gatifloxacin at 241 nm

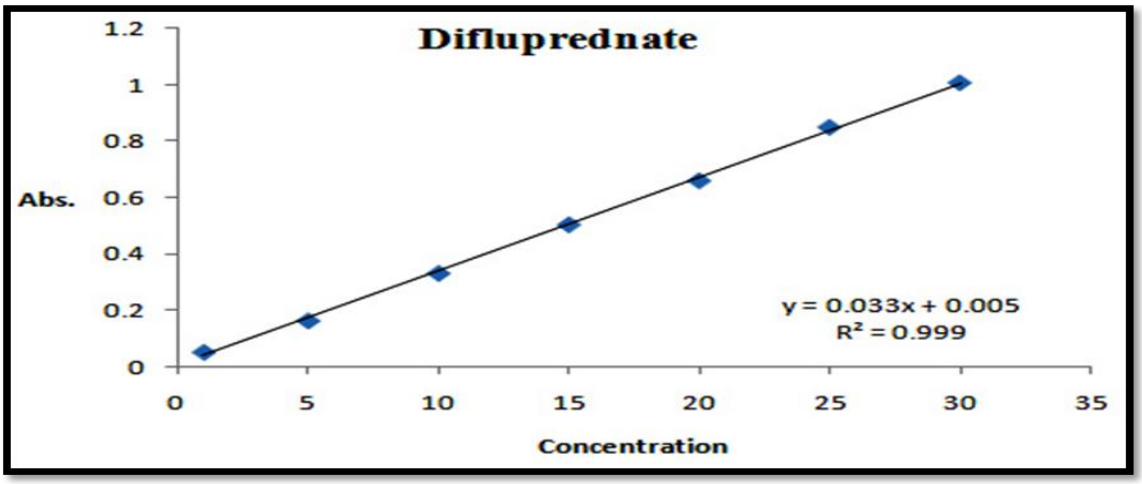

Fig.6 Calibration curve of difluprednate

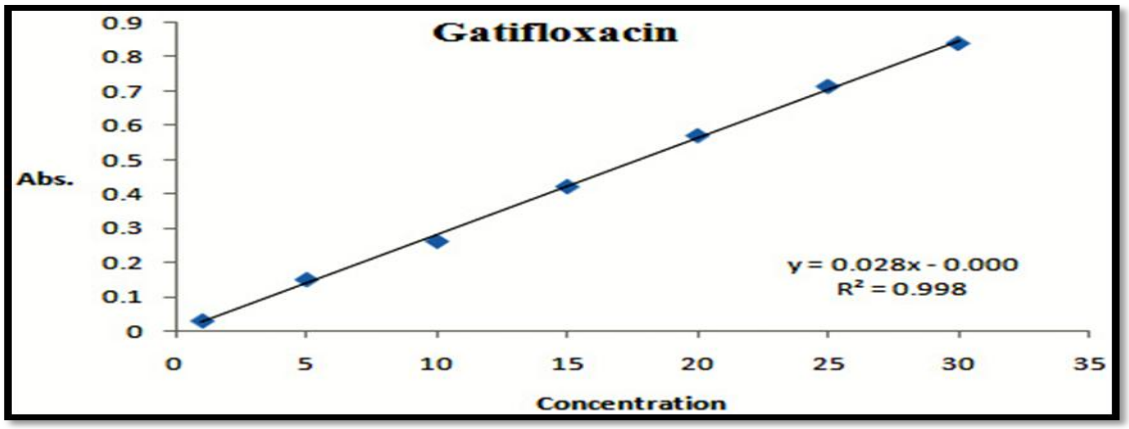

Fig.7 Calibration curve of gatifloxacin

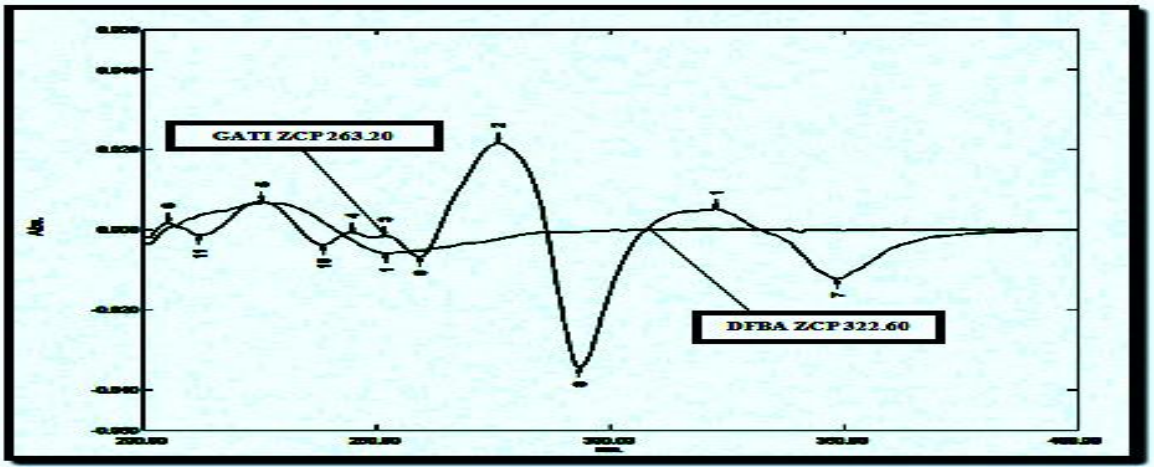

Fig.8 Overlain first-order derivative spectra of difluprednate and gatifloxacin

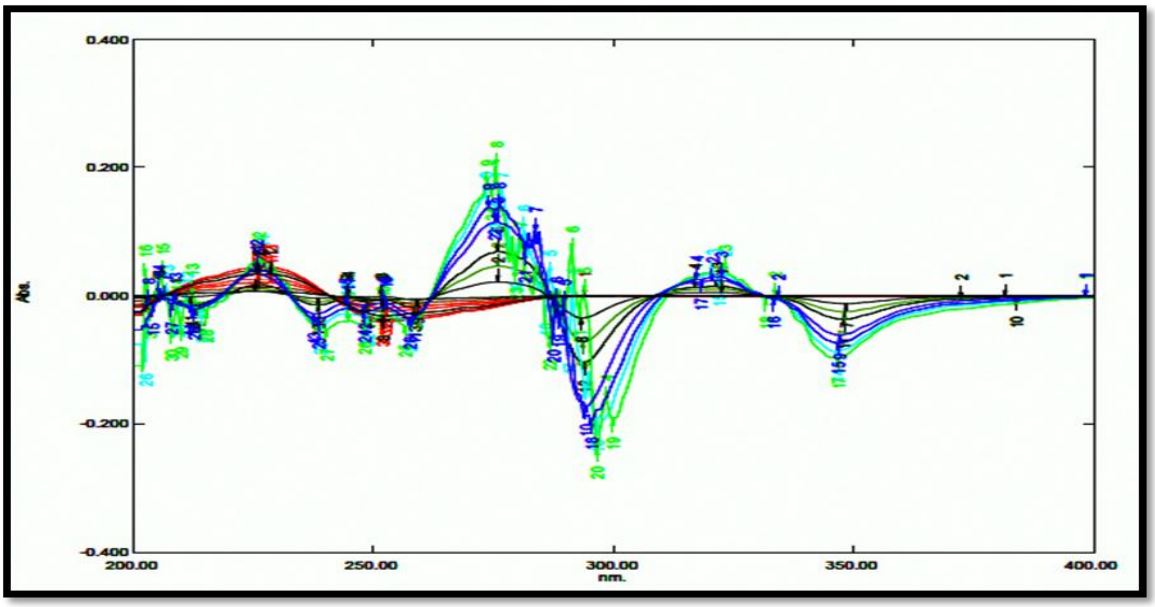

Fig.9 Overlain spectra of DFBA and GATI

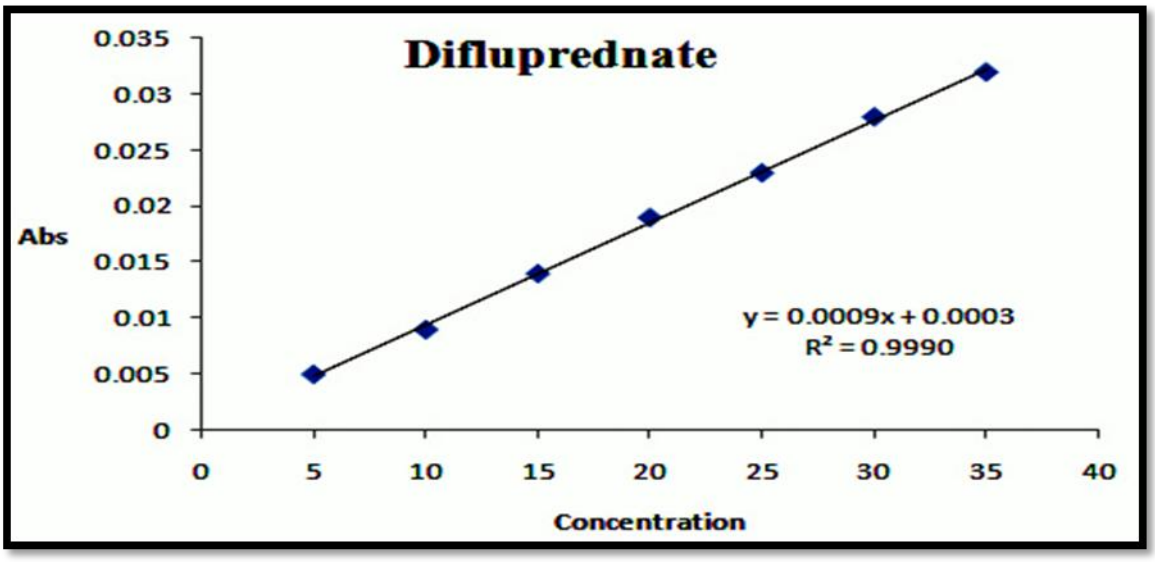

Fig.10 Calibration curve of Difluprednate

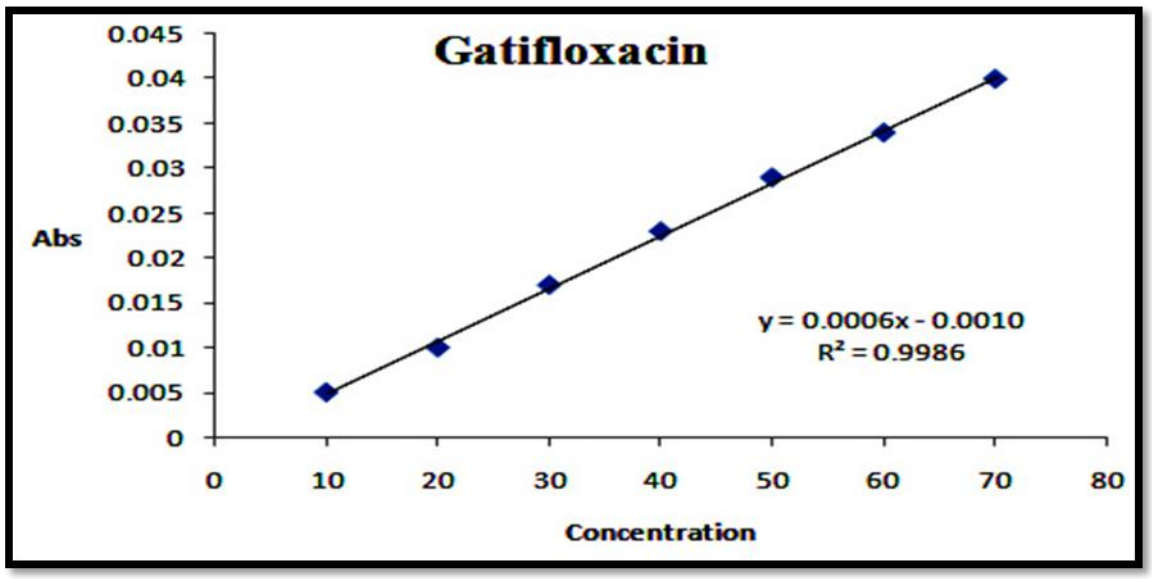

Fig.11 Calibration curve of Gatifloxacin

VI. CONCLUSION

Based on the results, obtained from the analysis of using described method, it can be concluded that the Q-absorption ratio method has linear response in the range of 1-30 µg/ml for the both drugs DFBA and GATI and First Order Derivative Method has linear response in the range of 5-35 µg/ml for DFBA and 10-70 µg/ml for GATI. The result of the analysis of synthetic mixture and formulation by the proposed methods are highly reproducible and reliable and is in good agreement with label claim of the drugs. The method can be used for the routine analysis of DFBA and GATI in combined dosage form.

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