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Research Article

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Simultaneous estimation of gatifloxacin and flurbiprofen sodium in ophthalmic formulation by UV-specrophotometric method

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ABSTRACT

A UV- Spectrophotometric method has been developed for the determination of Gatifloxacin and Flurbiprofen Sodium in their pharmaceutical formulation. The wavelength method involved solving simultaneous equations based on measurement of absorbance at two maximum wavelengths 245 nm and 293 nm for Flurbiprofen Sodium and Gatifloxacin respectively. The method was validated for various parameters according to ICH guideline. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 6 – 18 µg/ml and 0.6 – 1.8 µg/ml and correlation coefficient was found to be 0.9984 and 0.9995 for Gatifloxacin and Flurbiprofen Sodium respectively.

Keywords: Gatifloxacin, Flurbiprofen Sodium, Simultaneous Equation Method, Validation, Ophthalmic Formulation.

INTRODUCTION

Gatifloxacin(GFC) is chemically 1-Cyclopropyl-6-fluoro- 8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid(Figure 1)[1,2].GFC is a fluoroquinolones family of synthetic broad-spectrum antibiotics, which eradicate bacteria by interfering with DNA replication. However, the fluoroquinolones are relatively ineffective against intracellular pathogens[3]. Flurbiprofen Sodium (FS) is chemically Sodium(±)-2-(2-fluoro-4-biphenylyl) propionate dehydrates (Figure 2)[4,5]. FS is a propionic acid derivative and Non - Steroidal Anti – Inflammatory Drugs (NSAIDs) with antipyretic and analgesic activity[6]. Gatifloxacin is combination with Flurbiprofen sodium is used for reduction of post – operative inflammatory condition of eye,when bacterial infection exists.

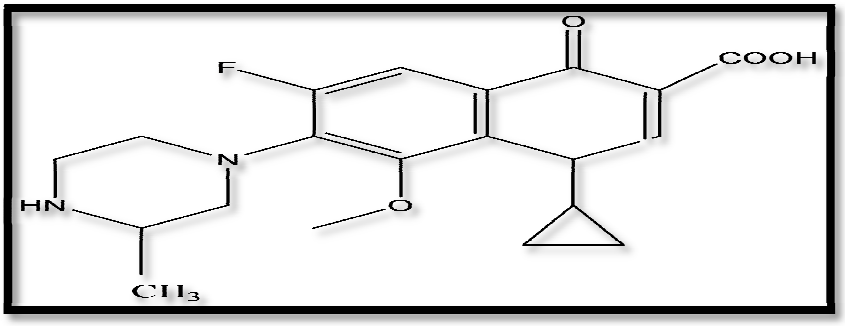

Figure 1: Chemical Structure of Gatifloxacin

In the literature, methods were described for the individual estimation of Gatifloxacinby Titrimetric[7], UV - Visible Spectrophotometry [8,9], RP- HPLC[10] and HPTLC[11]and for Flurbiprofen Sodium by Titrimetric[12], HPTLC[13]and RP- HPLC[14]methods. The methods were also given for simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium combine with other drugs by UV- Visible Spectrophotometry [15, 16], RP- HPLC [17-22] and HPTLC [23, 24] methods. Literature survey dose not reveal simultaneous determination of these drugs in their combined

pharmaceutical formulation and has not been reported in official pharmacopoeia.Therefore, it was thought of interest to develop and validate the RP- HPLC method for Gatifloxacin and Flurbiprofen Sodium in their ophthalmic formulation.

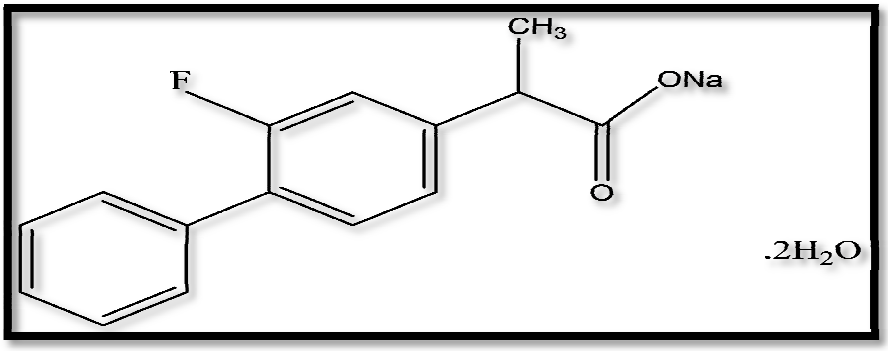

Figure 2: Chemical Structure of Flurbiprofen Sodium

EXPERIMENTAL SECTION

Instruments: UV–Visible Spectrophotometer (Agilent Technologies 8453), Sonicator–Ultrasonic (PCI Analytics), Sartorous Analytical weighing balance (PA 225 D)

Chemicals: GFC and FS bulk powder were procured as a gift samples from Yash Laboratories, Chikhali,Gujarat. , AR grade Methanol(S. D. Fine Chem Limited, Mumbai), and commercial pharmaceutical preparation FLUBIGAT Eye Drops, Mfg. by, EntodPharma is claimed to contain 0.3 % w/v of Gatifloxacin and 0.03 % w/v of Flurbiprofen Sodium.

Method Development

Overlain Spectra: Stock solution of GFC (9µg/ml) and FS (0.9µg/ml) were prepared in methanol for the selection of wavelength. The spectrum scans in range of 200 - 400 nm.

Based upon overlain spectra, the conclusion is that both drugs are shown absorbance on other $\lambda_{max.}$

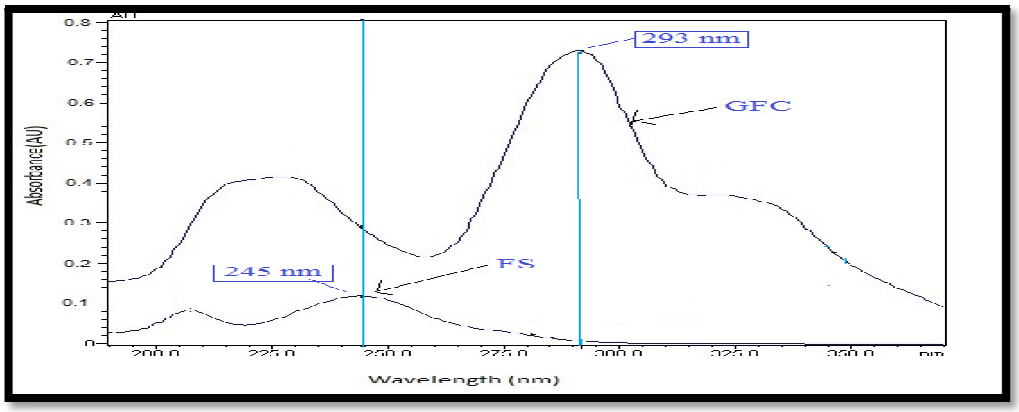

Figure 3 : UV overlain spectra of Gatifloxacin and Flurbiprofen Sodium

Preparation of Standard stock solution from bulk drugs:

An accurately weighed 30 mg of standard GFC powder and 30 mg of standard FS powder transfer in to 100 ml of separate volumetric flask and dissolve with methanol to getconcentration 300 µg/ml solution for GFC and 300µg/ml solution for FS. Take 5ml of that solution diluted with 50 ml of methanol and get final concentration 30µg/ml for GFC and 30µg/ml for FS. Standard solutions were prepared by dilution of stock solution with methanol to give the final concentration range of 6 – 18 µg/ml and 0.6 – 1.8 µg/ml for GFC and FS respectively.

Sample preparation: (Label claim: 0.3 % w/v Gatifloxacin and 0.03 % w/v Flurbiprofen Sodium). Take 1 ml sample and dilute with mobile phase in to 10 ml volumetric flask (30µg/ml of Gatifloxacin and 3µg/ml of Flurbiprofen Sodium). Take further 4 ml in 10 ml volumetric flask to get concentration12 µg/ml for GFC and 1.2 µg/ml for FS.

Method Validation [25]:

1. Linearity :

Five working standard solutions of each analytein the concentration range 6 - 18µg/ml for GFC and 0.6 – 1.8 µg/ml for FS were prepared and analysed (n=5).Calibration curve was constructed by plotting the absorbance against concentration using linear regression analysis.R^2 value was found 0.9984 for GFC and 0.9995 for FS. (Table 1)

2. Limit of Detection and Limit of Quantification:

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on standard deviation of the response and slope of the calibration curve. The LOD and LOQ for GFC and FS were found. (Table 1)
LOD and LOQ are calculated by formula:

LOD = 3.3 x σ / s and LOQ = 10 x σ / s
Where, σ= standard deviation of the y- intercepts of the regression line.
s = slope of the calibration curve.

3. Precision :

Method precision:The precision of the method was evaluated by inter-day and intra-day precision. For intra-day precision, three different concentrations of GFC (6, 12 and 18 µg/ml) and FS (0.6, 1.2 and 1.8µg/ml) were prepared in triplicate and analysed during same day. For Inter- day precision the same concentrations were analysed different day. The % RSD values were calculated. (Table 2)

System precision: The Precision of system was evaluated by repeatability. This was analysed by repeated analysedof six sample solution of GFC (12µg/ml) and FS (1.2 µg/ml)under same condition. The % RSD was calculated for both analyte.The % RSD values werecalculated. (Table 3)

4. Accuracy:

The accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amount of GFC and FS to a sample solution of known concentration.The percentage of recovery was calculated from the amount of drug found in the solution. (Table 4)

5. Assay of the marketed formulation:

The developed method was applied to the simultaneous estimation of GFC and FS in ophthalmic formulation. (Table 5)

RESULTS AND DISCUSSION

SIMULATENOUS EQUATION METHOD:

The developed method was applied for simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in ophthalmic formulation by SIMULTANEOUS EQUATION METHOD.

Equation:
$C_x = A_2a_{Y1} – A_1a_{Y2} / a_{x2}a_{Y1} – a_{x1}a_{Y2}$
$C_Y = A_1a_{x2} – A_2a_{x1} / a_{x2}a_{Y1} – a_{x1}a_{Y2}$

Where,
a_{x1} (absorptivity of Gatifloxacin at 293 nm) = 968.89
a_{x2} (absorptivity of Gatifloxacin at 245 nm) = 333.76
a_{Y1} (absorptivity of Flurbiprofen Sodium at 293 nm) = 50.05
a_{Y2} (absorptivity of Flurbiprofen Sodium at 245 nm) = 903.80

Validation:

1. Linearity:

Calibration graphs were constructed by plotting the absorbencies versus their corresponding concentrations. Good linearity was obtained in the range6 - 18µg/ml for GFC and 0.6 – 1.8µg/ml for FS. The results are shown in Table 1. LOD and LOQ were calculated from slope and standard deviation y- intercepts of the regression line of the calibration curve shown in figure 7 and 8.

Table 1: Linearity Data by Regression Analysis (n = 5)

Parameters	GFC (293 nm)	FS (245 nm)
Concentration range	6 – 18µg/ml	0.6 – 1.8 µg/ml
Regression equation	y = 39.383x - 72.803	y = 310.75x - 38.467
R^2 Value	0.9988	0.9992
LOD	1.45	0.028
LOQ	4.39	0.286

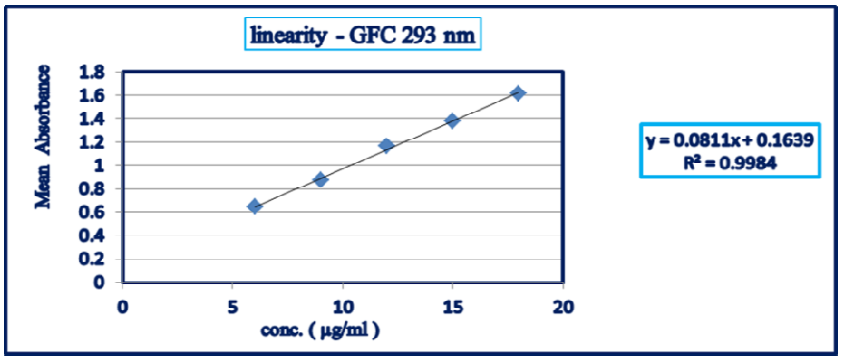

Figure 4: Calibration curve of Gatifloxacin at 293 nm

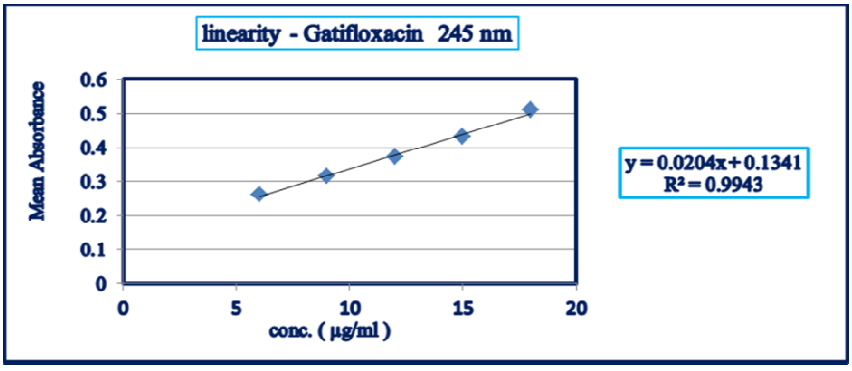

Figure 5: Calibration curve of Gatifloxacin at 245 nm

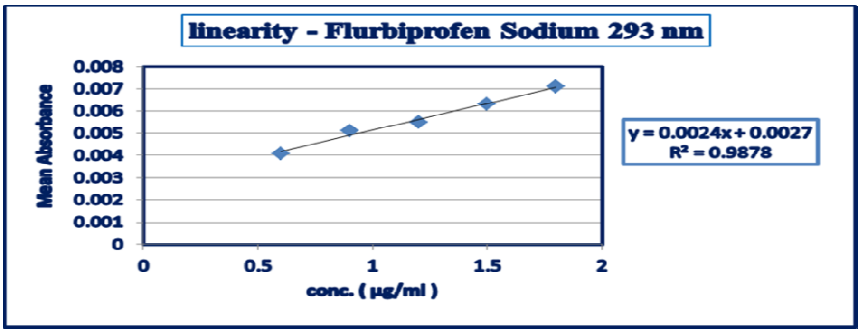

Figure 6: Calibration curve of Flurbiprofen Sodium 293 nm

2. Precision:

The precision of method and system were evaluated and % RSD values were calculated. The precision of the method was satisfactory. The results are shown in Table 2 and 3.

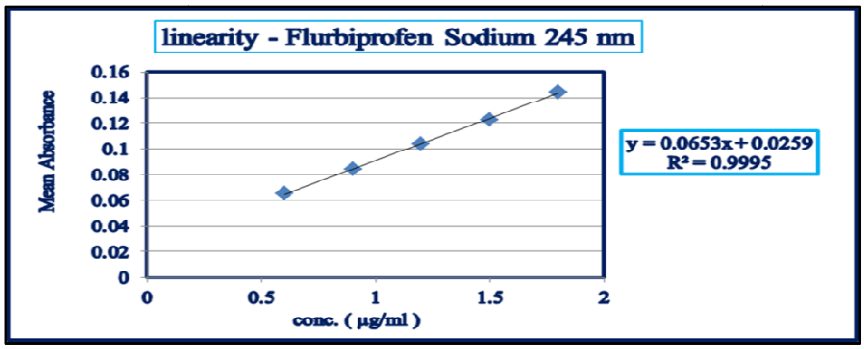

Figure 7: Calibration curve for Flurbiprofen Sodium at 245 nm

Table 2: **Intra-day and Inter-day precision Data (n=3)**

Conc. (µg/ml)	Intra – day Precision (% RSD)		Inter – day Precision (% RSD)	
	293 nm	245 nm	293 nm	245 nm
GFC				
6	0.26	0.51	0.23	0.58
12	0.16	0.82	0.13	0.40
18	0.14	0.73	0.09	0.29
FS				
0.6	0.14	0.98	0.15	1.25
1.2	0.14	0.43	0.14	0.99
1.8	0.23	0.32	0.10	1.32

Table 3: **Repeatability Data (n=6)**

Conc. (µg/ml)	% RSD	
	293 nm	245 nm
12	0.12	0.26
1.2	0.99	0.15

3. Accuracy :

The accuracy of the method was determined by recovery studied. These studies carried out at 80 %, 100 % and 120 % level. The results are shown in Table 4.

Table 4: Recovery Studies Data

Drugs	Amount taken (µg/ml)	% Added	Mean % Recovery ± SD (n=3)
GFC	6	80	101.26 ± 0.13
		100	101.52 ± 0.04
		120	100.12 ± 0.04
FS	0.6	80	99.99 ± 0.19
		100	99.55 ± 0.12
		120	99.75 ± 0.43

Table 5: Analysis Data of Marketed formulation(n=5)

Label claim		Amount taken (µg/ml)		Mean Amount found (µg/ml)		Mean % Assay	
GFC	FS	GFC	FS	GFS	FS	GFC	FS
0.3 % w/v	0.03 % w/v	12	1.2	12.04	1.20	100.31	100.16

CONCLUSION:

The developed Spectrophotometric method was suitable for the simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium as bulk and marketed formulation without any interfering from the excipients. The developed method was validated for the various parameters as per ICH guideline.

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