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DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND PREDNISOLONE ACETATE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economic dual wavelength method for simultaneous estimation of Ofloxacin and Prednisolone acetate in pharmaceutical formulation. The principle of dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". Ofloxacin was determined directly at 323.40 nm in methanol. The wavelengths selected for determination of Prednisolone acetate were 323.40 nm and 277 nm in methanol. The two drugs follow Beer-Lambert's law over the concentration range of 3-40 µg/ml. The method was successfully applied to pharmaceutical formulation. The suitability of this method for the quantitative determination of Ofloxacin and Prednisolone acetate was proved by validation. The results of analysis have been validated statistically and by recovery studies according to ICH guidelines.

KEYWORDS: Ofloxacin, Prednisolone acetate, Dual wavelength method, Pharmaceutical formulation, Spectrophotometric, Validation

INTRODUCTION

Ofloxacin (OFL) **(**Figure 1**)** is chemically, 9-fluro-2-3 dihydro-3-methyl-10- (4-methyl 1-piperazinyl)-7-oxo-7H- pyrido [1, 2, 3-de] 1, 4 benzoxazine-6-carboxylic acid.[1], is a

fluoroquinolone antibacterial agent used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy.[2]

This drug is official in Indian Pharmacopoeia (IP).[3], United State Pharmacopoeia (USP).[4], Europian Pharmacopoeia (EP).[5], British Pharmacopoeia (BP).[6], Japanese Pharmacopoeia (JP).[7]. IP describe LC, USP describe LC and potentiometry and, EP, BP, and JP describe potentiometric method for the estimation. Literature survey revels that HPLC.[8-11], HPTLC.[12], spectrophotometry.[13,14], capillary Electrophoresis.[15] methods for determination of OFL in single and combination with other drugs. Prednisolone acetate (PRD) **(**Figure 2**)** is chemically, 11β 17, 21-trihydroxypregna-1, 4- diene-3, 20-dione 21-acetate.[16], is a hydrocortisone type corticosteroid. It is used for infections of the eye [17]. This drug is official in IP. [18], USP.[19], EP. [20], BP.[21] and JP .[22]. IP, USP, EP, BP and JP describe LC method for the estimation. Literature survey revels that HPLC.[23-26], spectrophotometry.[27,28] methods for determination of PRD in single and combination with other drugs. The combination of OFL and PRD is not official in any pharmacopeia; hence no official method is available for estimation of these two drugs in combined dosage form. Literature survey revels that several HPLC.[29-31], HPTLC.[32] and spectrophotometry.[33] methods have been reported for estimation of OFL and PRD in combined dosage form. Literature survey reveals only single spectrophotometric method based on simultaneous equations for estimation of these two drugs in mixture; hence it is thought of interest to develop and validate alternative spectrophotometric method for simultaneous estimation of OFL and PRD in combined dosage form. The present manuscript describe alternative simple, sensitive and cost effective spectrophotometric method based in dual wavelength spectrophotometric method for the simultaneous estimation of OFL and PRD in combined ophthalmic formulation.

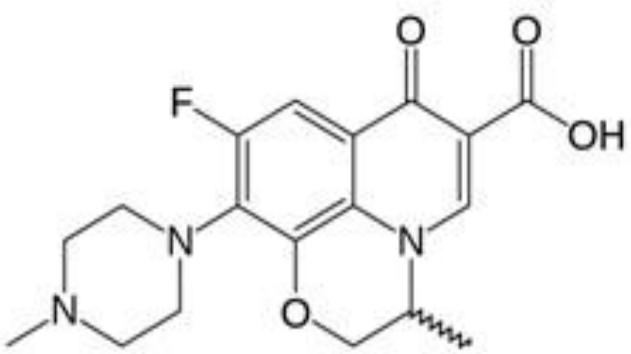

FIGURE 1: CHEMICAL STRUCTURE OF OFLOXACIN (OFL)

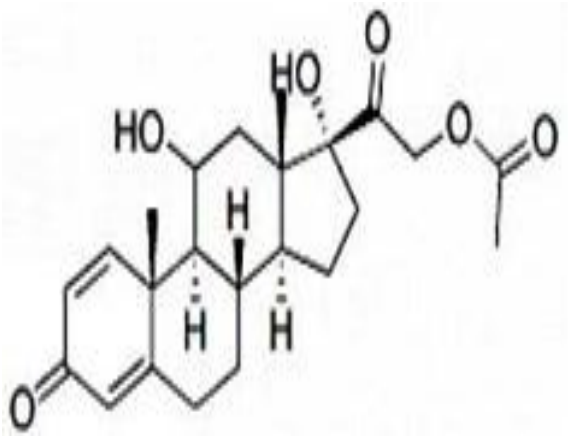

FIGURE 2: CHEMICAL STRUCTURE OF PREDNISOLONE ACETATE (PRD)

MATERIALS AND METHODS

Reagents and Materials

Pure sample of OFL was provided as a gift sample from Camper Healthcare, Ganpat University, Mahesana-Gozaria highway, Kherva, Gujarat. PRD was obtained from Maharshi Pharma Chem Private Limited Kheda (Nadiad), Gujarat, India. A commercial ophthalmic formulation (Ocepred ophthalmic suspension) was obtained from local market, Methanol AR Grade was received form S.D fine Chemicals Ltd, Mumbai, India. Whatman filter paper no 41. All the chemicals used were of analytical grade.

Apparatus

The instruments used for the present study were an UV-visible double beam spectrophotometer (Shimadzu, 1800, Japan) having spectral width of 2 nm, wavelength accuracy of ± 0.5 nm and pair of 10 mm matched quarts cell was used to carry out spectrophotometric measurements. Spectra were automatically recorded by UV-probe 2.0 system software. An electronic analytical balance (Sartorius CP224S) and an ultrasonic bath were also used during experiment.

Preparation of standard stock solutions

Standard stock solution of OFL and PRD (100 µg/ml) was prepared separately by dissolving an accurately weighed quantity of OFL (10 mg) and PRD (10 mg) to a separate 100 ml volumetric flask and diluted up to mark with methanol to obtained standard solution having concentration of 100 µg/ml for both drugs.

Development of method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest, independent of the interfering components. From the overlain spectra of two drugs (Figure 3), it is evident that direct determination of OFL at 323.40 nm (no absorbance of PRD at 323.40 nm). For estimation of PRD, two wavelengths selected (323.40 nm and 277 nm) where OFL shows same absorbance. The determination of PRD is carried out by subtracting absorbance due to OFL at 277 nm and the difference between 277 nm and 323.40 nm is directly proportional to concentration of PRD in the mixture.

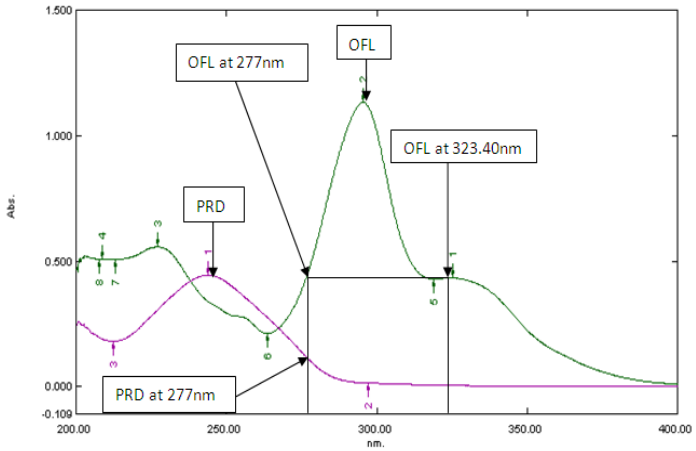

Figure 3: Overlain spectra of OFL (10 µg/ml) and PRD (10 µg/ml) in methanol

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[34]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 3-40 µg/ml for each OFL and PRD. Accurately measured standard stock solutions of each OFL and PRD (0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of solution were then measured at 277 nm and 323.40 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions (n = 6) of OFL and PRD (20 µg/ml for both drugs) without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of OFL and PRD (10, 20 and 30 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% recovery study)

The accuracy of the method was determined by calculating recoveries of OFL and PRD by the standard addition method. Known amounts of standard solutions of OFL and PRD were added at 50%, 100% and 150% level to prequntified sample solutions of OFL (3 µg/ml) and PRD (10 µg/ml). The amounts of OFL and PRD were estimated by putting obtained values in the respective regression line equation. The experiment was repeated three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug was 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

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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

Determination of OFL and PRD in their combined ophthalmic formulation

The eye drop (1.0 ml) containing 0.01 gm of OFL and 0.003 gm of PRD was transferred to 25 ml volumetric flask. Methanol (10 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of PRD and 3 µg/ml of OFL. The resulting solution was analyzed by proposed method.

RESULTS AND DISCUSSION

In this method, two specific wavelengths are selected, first wavelength λ1 at which reasonable absorbance of OFL was observed and there was no interference of PRD at this wavelength (323.40 nm). Second wavelength λ2 was the wavelength at which the absorbance of OFL was same as at λ1, and PRD was also having some absorbance at this wavelength (277 nm). The absorbance at these two wavelengths was found to be equal for OFL. These two selected wavelengths were employed to determine the concentration of PRD from the mixture of OFL and PRD. The difference in absorbance at these two wavelengths (A277–A323.40) cancels out the contribution of absorbance of OFL in formulation.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economical for the routine simultaneous estimation of two drugs. The linearity ranges for both drugs were found to be 3-40 µg/ml. Characteristic parameters of regression equation and correlation are given in (Table 1). Precision was calculated as repeatability (% RSD) and intra and inter-day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery, and the mean was determined (Table 2). The LOD and LOQ were found to be 0.43 and 1.29 µg/ml, respectively for OFL and 0.86 and 2.63 µg/ml, respectively for PRD, indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of OFL and PRD present in binary mixture. The results obtained are in good agreement with the corresponding labeled amount (Table 3). By observing the validation parameters, the method was found to be sensitive, accurate and precise. Hence, the method can be employed for the routine analysis of these drugs in combination.

Table 1: Regression analysis data and summary of validation parameters for the proposed Method

PARAMETERS	OFL	PRD
Wavelength range (nm)	323.40	277 and 323.40
Beer's law limit (µg/ml)	3 – 40	3 – 40
Regression equation (y = mx+c) Slope (m) Intercept (c)	Y = 0.0435x – 0.0143 0.0435 0.0143	Y = 0.0099x – 0.0029 0.0099 0.0029
Correlation Coefficient (r^2)	0.9994	0.9997
Method precision (Repeatability) (% RSD),(n= 6)	0.31	0.74
Intraday (n = 3) (% RSD)	0.35 – 0.62	0.67 – 1.04
Interday (n = 3) (% RSD)	0.46 – 0.97	1.19 – 1.59
LOD (µg/ml)	0.43	0.86
LOQ (µg/ml)	1.29	2.63
Accuracy (Mean % Recovery ± S.D.) (n=3)	99.81 ± 0.63	99.99 ± 0.67

% Assay ± S.D. (n = 5)	100.8 ± 0.76	100.2 ± 0.58

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification

SD = Standard deviation, n = number of replicates.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
OFL	I	3	50	100.3 ± 0.88
	II	3	100	99.44 ± 0.69
	III	3	150	99.66 ± 0.33
PRD	I	10	50	99.55 ± 0.50
	II	10	100	100.1 ± 0.84
	III	10	150	100.3 ± 0.67

S.D. is Standard deviation and n is number of replicate

Table 3: Analysis of OFL and PRD by proposed method

Formulation	Label claim		Amount found		% Label claim ± S. D. (n = 5)	
Ophthalmic formulation	OFL	PRD	OFL	PRD	OFL	PRD
	3	10	3.02	10.0	100.8 ± 0.76	100.2 ± 0.58

S.D. is Standard deviation and n is number of replicates.

CONCLUSION

The proposed dual wavelength spectrophotometric method was found to be simple, sensitive, accurate, precise and economical and can be employed for the routine analysis of these two drugs in combined pharmaceutical preparation.

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