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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF PREDNISOLONE ACETATE AND OFLOXACIN IN EYE-DROP

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of prednisolone acetate and ofloxacin in combined eye drop dosage form. The method is based on the simultaneous equations method for analysis of both the drugs using methanol as solvent. Prednisolone acetate has absorbance maxima at 243 nm and ofloxacin has absorbance maxima at 228 nm in methanol. The linearity was obtained in the concentration range of 5-17 µg/ml and 1-13 µg/ml for prednisolone acetate and ofloxacin respectively. The concentrations of the drugs were determined by using simultaneous equations method at both the wavelength set 243nm for PRD and 228nm for OFL. The method was successfully applied to pharmaceutical dosage form because no interference from the eye-drop excipients was found. The suitability of this method for the quantitative determination of prednisolone acetate and ofloxacin was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of prednisolone acetate and ofloxacin in pharmaceutical dosage form. The result of analysis has been validated statistically and by recovery studies.

Keywords:

Acetate,
Ofloxacin,
Simultaneous equation method,
Eye-drop,
Validation

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INTRODUCTION: Prednisolone acetate (PRD) (**Figure 1**) is chemically, 11β 17,21-trihydroxypregna-1,4- diene-3,20-dione 21-acetate', is a hydrocortisone type corticosteroid¹. It is used for infections of the eye^[2]. Prednisolone acetate is official in B.P.³ and E.P. BP and E.P. describe liquid chromatography method for its estimation. Literature survey reveals RP-HPLC^{4, 5} and spectrophotometric. methods for determination of PRD with other drugs.

Ofloxacin (OFL) (**Figure 2**) 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 acid⁶, is a fluoroquinolone antibacterial agent used in

the treatment of chlamydia or chlamydia infections including nongonococcal urethritis and in mycobacterial infections such as leprosy⁷. It is official in IP, BP and USP. IP⁸, BP⁹ and USP describe potentiometric method for its estimation.

Literature survey reveals first derivative fluorescence spectroscopy¹⁰, HPLC with fluorescence detector for estimation of ofloxacin in human plasma¹¹. Literature survey also reveals spectrophotometric¹², RP-HPLC and HPTLC¹³ methods for determination of OFL with other drugs. The combined dosage forms of PRD and OFL are available in the market and used in eye infection.

The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of PRD and OFL in their combined dosage forms. Literature survey does not reveal any simple spectro-photometric or chromatographic method for simultaneous estimation of PRD and OFL in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation for estimation of both drugs in their combined eye drop dosage forms.

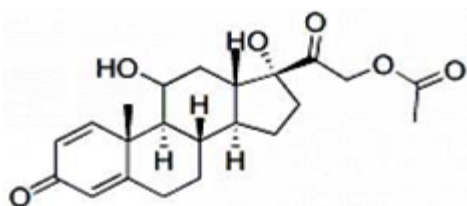


FIGURE 1: CHEMICAL STRUCTURE OF PREDNISOLONE ACETATE (PRD)

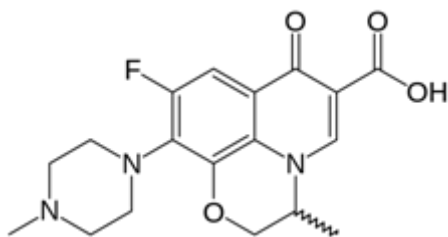


FIGURE 2: CHEMICAL STRUCTURE OF OFLOXACIN(OFL)

MATERIALS AND METHODS:

Apparatus

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). An electronic analytical balance (Acculab), and an ultrasonic bath was used in the study.

Reagents and Materials: PRD and OFL bulk powder was gifted by Corona Remedies Pvt. Ltd., Ahmedabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S.D. Fine Chemicals Ltd., Mumbai, India.

Preparation of Standard Stock Solution: An accurately weighed quantity of PRD (10 mg) and OFL (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of PRD (100 µg/ml) and OFL (100 µg/ml).

Method:

Simultaneous Equation Method: In simultaneous equation method, five working standard solutions having concentration 5, 7, 9, 11, 13 µg/ml for PRD and seven working standard solutions having concentration 1, 3, 5, 7, 9, 11, 13 µg/ml for OFL were prepared in methanol and the absorbance at 243 nm (λ -max of PRD) and 228 nm (λ -max of OFL) were measured and absorptivity coefficients were calculated using calibration curve.

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

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \dots \dots \dots (1)$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \dots \dots \dots (2)$$

Where A_1, A_2 are absorbance of mixture at 243 nm (λ_1) and 228 nm (λ_2) respectively, a_{x1} and a_{x2} are absorptivities of PRD at λ_1 and λ_2 respectively, a_{y1} and a_{y2} are absorptivities of OFL at λ_1 and λ_2 respectively, C_x and C_y are concentrations of PRD and OFL respectively.

Validation of Proposed Method:

Linearity (Calibration curve): The calibration curves were plotted over a concentration range of 5-13 µg/ml for PRD and 1-13 µg/ml OFL. Accurately measured standard stock solutions of each PRD (0.5, 0.7, 0.9, 1.1, 1.3 ml) and OFL (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3) 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 243 nm and 228 nm.

The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Precision:

- **Intraday:** Mixed solution containing 3-10 µg/ml of both was analyzed three times on the same day and %R.S.D was calculated.
- **Interday:** Mixed solution containing 3-10 µg/ml of both was analyzed on three different days and %R.S.D was calculated.

Accuracy: Accuracy was determined by calculating recovery of PRD and OFL by the standard addition method. From working sample solution of test (100 µg/ml of both), 1 ml of solution were taken and increasing aliquots of combined working standard solution (0.8, 1.0 and 1.2 ml from 100 µg/ml of both) were added and diluted to 10 ml with methanol. These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for PRD and OFL.

The amount of PRD and OFL was calculated at each level by simultaneous equation method and absorbance correction method and % recoveries were computed.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and the limit of quantitation (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.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

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

Analysis of PRD and OFL in combine Eye drop: The eye-drop equivalent to 0.003 gm of PRD and 0.01 gm of OFL were weighed and transferred to 100 ml volumetric flask. Methanol (50 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 absorbances of the sample solution i.e. A1 and A2 were recorded at 243 nm (λ -max of PRD) and 228 nm (λ -max of OFL), respectively, Relative concentration of two drugs in the sample was calculated using above equation (1) and (2).(3),(4).

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

Parameters	PRD	OFL
Wavelength range (nm)	243	228
Beer's law limit (µg/ml)	5-13	1-13
Regression equation ($y = mx + c$)	$y = 0.041x + 0.009$	$y = 0.1024x + 0.0232$
Slope	0.041	0.1024
Intercept	0.009	0.0232
Correlation Coefficient (r^2)	0.999	0.9996
System Precision (%R.S.D) ^a		
1. Intraday Precision (n = 3)	0.069-0.2013%	1.073-1.96%
2. Interday Precision (n = 3)	0.684-1.810%	0.565-1.99%
Accuracy (% recovery) (n = 3)	99.98-101.35%	98.11-101.44%
LOD ^b (µg/ml)	0.64	0.1638
LOQ ^c (µg/ml)	1.957	0.4965
Assay (\pm R.S.D) ^a (n = 3)	100.8 \pm 0.84	97.6 \pm 1.47

^aRSD = Relative standard deviation. ^bLOD = Limit of detection. ^cLOQ = Limit of quantitation and n is number of replicates

RESULT AND DISCUSSION: In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength¹⁶, which was fulfilled in case of both these drugs. The two

wavelengths were used for the analysis of the drugs were 243 nm (λ -max of PRD) and 228 nm (λ -max of OFL) and at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of PRD (243 nm) and OFL (228 nm)

in methanol is shown in **Figure 3**. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (**Table 2**). The method was successfully used to determine the amounts of PRD and OFL present in the

eye drop dosage forms. The results obtained were in good agreement with the corresponding labeled amount (**Table 3**). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs.

TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken ($\mu\text{g/ml}$)	Amount added ($\mu\text{g/ml}$)	Amount added (%)	% Mean recovery ($\pm\text{S.D.}$) (n = 3)
PRD	I	10	8	80	100.49 \pm 1.74
	II	10	10	100	99.98 \pm 1.18
	III	10	12	120	101.35 \pm 1.36
OFL	I	3	2.4	80	98.11 \pm 0.43
	II	3	3	100	98.17 \pm 0.86
	III	3	3.6	120	101.44 \pm 1.12

R.S.D is Relative standard deviation and n is number of replicates.

TABLE 3: ANALYSIS OF PRD AND OFL BY PROPOSED SIMULTANEOUS METHOD.

Tablet	Labelled claim (mg/ml)		Amount found (mg/ml)		% Label claim ($\pm\text{R. S. D.}$) (n = 3)	
	PRD	OFL	PRD	OFL	PRD	OFL
I	10	3	10.41	2.813	100.8 \pm 0.84	97.6 \pm 1.47

R.S.D. is Relative standard deviation and n is number of replicates.}

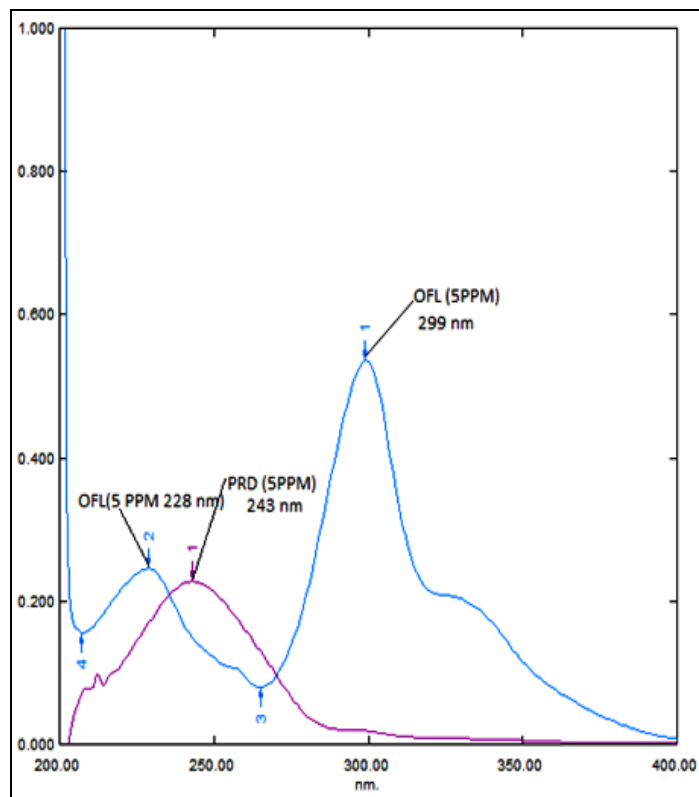


FIGURE 3: OVERLAIN ABSORPTION SPECTRA OF PRD (243 nm) AND OFL (228 nm and 299 nm) IN METHANOL

CONCLUSION: The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of PRD and OFL. The developed method was validated as per ICH guidelines. Statistical analysis proved that the

method is repeatable and selective for the analysis of PRD and OFL in combination as a single drug in bulk as well as in pharmaceutical formulations.

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