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## Spectrophotometric Estimation of Ofloxacin and Prednisolone Acetate in Binary Mixture

	
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### ABSTRACT

A simple and sensitive spectrophotometric method based on Q-absorbance ratio was developed for the simultaneous estimation of Ofloxacin and Prednisolone acetate in combined pharmaceutical formulation. Q-absorbance ratio method based on formation of Q-absorbance equation at two wavelengths, one is isoabsorptive point and another is the  $\lambda_{\text{max}}$  of one of the two drugs. Absorbance was measured at two selected wavelengths, one is 236.80 nm (isoabsorptive point) and another being 243 nm ( $\lambda_{\text{max}}$  of Prednisolone acetate). The two drugs comply with Beer's Lambert's law over the linearity range 3-30  $\mu\text{g/ml}$ . The method was validated as per International Conference on Harmonization guideline rules in terms of linearity, accuracy (recovery study), precision (repeatability, intraday, interday validation), limit of detection, limit of quantification. All the validation parameters were found to be within acceptable limits. The method was found to be simple, sensitive, rapid, cost effective, accurate, and precise for the routine analysis of both the drugs in the binary mixture.



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## INTRODUCTION

Ofloxacin (OFL) (Figure 1) is chemically, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de] 1, 4-benzoxazine-6-carboxylic acid<sup>1</sup>, is a fluoroquinolone antibacterial agent used in the treatment of chlamydia infections including nongonococcal urethritis and in mycobacterial infections such as leprosy<sup>2</sup>.

This drug is official in Indian Pharmacopoeia (IP)<sup>3</sup>, United State Pharmacopoeia (USP)<sup>4</sup>, European Pharmacopoeia (EP)<sup>5</sup>, British Pharmacopoeia (BP)<sup>6</sup> and Japanese Pharmacopoeia (JP)<sup>7</sup>. IP describe LC, USP describe LC and potentiometry and EP, BP, and JP describes potentiometric method for its estimation. Literature survey reveals HPLC<sup>8-11</sup>, HPTLC<sup>12</sup>, spectrophotometry<sup>13,14</sup>, capillary electrophoresis<sup>15</sup> methods for determination of OFL in single and combination with others drugs. Prednisolone acetate (PRD) (Figure 2) is chemically, 11 $\beta$ -17, 21-trihydroxypregna-1,4-diene-3,20-dione 21-acetate<sup>16</sup>, is a hydrocortisone type corticosteroid. It is used for infections of the eye<sup>17</sup>. This drug is official in IP<sup>18</sup>, USP<sup>19</sup>, EP<sup>20</sup>, BP<sup>21</sup> and JP<sup>22</sup>. IP, USP, EP, BP and JP describe LC method for its estimation. Literature survey reveals HPLC<sup>23-26</sup>, spectrophotometry<sup>27,28</sup> methods for determination of PRD in single and combination with other drugs. The combination of OFL and PRD is not official in any pharmacopoeia; hence no official method is available for estimation of these two drugs in combined dosage form. Literature survey reveals HPLC<sup>29-31</sup>, HPTLC<sup>32</sup> and spectrophotometry<sup>33</sup> methods 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 on Q-absorbance ratio for the simultaneous estimation of OFL and PRD in combined ophthalmic formulation.

## MATERIAL AND METHODS

### Instruments

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  $\pm 0.5$  nm and pair of 10 mm matched quartz 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.

### Materials and Reagents

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

### Preparation of Standard Stock Solution

Standard stock solution was prepared by accurately weighing 10 mg of OFL and PRD in separate 100 ml calibrated volumetric flask and made up the volume with methanol up to 100 ml to obtained concentration of 100 µg/ml for both the drugs.

### Methodology



Q - absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the  $\lambda_{max}$  of one of the two drugs. From the overlay spectra of two drugs, it is observed that OFL and PRD show an isoabsorptive point at 236.80 nm. The second wavelength chosen is 243 nm, which is the  $\lambda_{max}$  of PRD. Working standard solutions having concentration 3, 4, 8, 12,16, 20, 24 and 30 µg/ml for OFL and PRD were prepared in methanol and the absorbances at 236.80 nm (isoabsorptive point) and 243 nm ( $\lambda_{max}$  of PRD) were measured and absorptivity coefficients were calculated using calibration curve.

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

$$CX = [(QM - QY) / (QX - QY)] \times A1/ax1 \dots \dots \dots (1)$$

$$CY = [(QM - QX) / (QY - QX)] \times A1/ay1 \dots \dots \dots (2)$$

Where, A1 and A2 are absorbances of mixture at 236.80 nm and 243 nm; ax1 and ay1 are absorptivities of OFL and PRED at 236.80 nm; ax2 and ay2 are absorptivities of OFL and PRED respectively at 243 nm;  $QM = A2 / A1$ ,  $QX = ax2 / ax1$  and  $QY = ay2 / ay1$ .

## **VALIDATION OF THE METHOD**

The method was validated as per the rules of International Conference on Harmonization (ICH) guidelines<sup>34</sup>.

### **Linearity (Calibration curve)**

The calibration curves were plotted over a concentration range of 3-30 µg/ml for OFL and PRD. Standard solution of each OFL and PRD (0.3, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 3.0 ml) were transferred in separate 10 ml flask and diluted with methanol. All absorbance were measured at 236.80 nm and 243 nm. Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

### **Precision**



### **Repeatability**

Repeatability of the method was determined by analyzing standard solution of OFL and PRD at (12 µg/ml for OFL and PRD) six times without changing the parameters of measurement and % RSD was calculated.

### **Intermediate Precision**

The intraday and interday precision of the proposed method was performed by analyzing the corresponding responses three times on the same day (intraday) and on three different days (interday) over a period of one week for three different concentrations (8, 16 and 24 µg/ml) of standard solutions of OFL and PRD.

### **Accuracy**

The accuracy of an analytical procedure is the closeness of agreement between the value which is accepted as true value and the value found. The recovery experiment was carried out by adding known amount of standard solution of OFL and PRD at 50%, 100%, and 150%

level to predetermined sample solution of OFL (3 µg/ml) and PRD (10 µg/ml). The amount of OFL and PRD were calculated by putting obtained values in the equation (1) and (2). The recovery study analysis was repeated three times and average recoveries were calculated.

### **Limit of Detection and Limit of Quantification**

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio by the use of standard deviation of the response and the slope of the calibration curve. The Limit of Detection and Limit of Quantification were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

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

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

Where,  $\sigma$  = the standard deviation of the response,

S = slope of the calibration curve.

### **Analysis 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 3 µg/ml of OFL and 10 µg/ml of PRD. The resulting solution was analyzed by proposed method.

### **RESULT AND DISCUSSION**

In Q - absorbance ratio spectrophotometry method, the foremost and prime need is that both the drugs should comply with the Beer's law at all the wavelength. OFL and PRD obeyed linearity in the concentration range of 3-30 µg/ml in methanol at their respective  $\lambda_{\text{max}}$  and isoabsorptive point with correlation coefficient ( $r^2 > 0.99$ ). The overlain absorption spectra of OFL and PRD showing isoabsorptive point in methanol is shown in Figure 3.

**Table 1: Result of recovery study of OFL and PRD by developed method**

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n=3)
OFL	I	3	50	99.88 ± 0.19
	II	3	100	99.44 ± 0.38
	III	3	150	99.66 ± 0.33
PRD	I	10	50	99.60 ± 0.52
	II	10	100	100.0 ± 0.25
	III	10	150	99.43 ± 0.40

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

**Table 2: Analysis of OFL and PRD in Ophthalmic formulation by developed method**

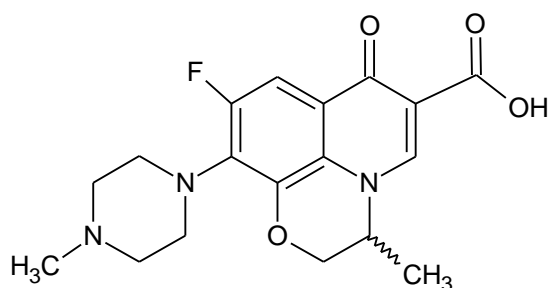
Formulation	Label claim (mg)		Amount found(mg)		% Label claim ± S.D (n=5)	
	OFL	PRD	OFL	PRD	OFL	PRD
Ocepred Ophthalmic suspension	3	10	3.013	10.016	100.4 ± 1.07	100.1 ± 0.76

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

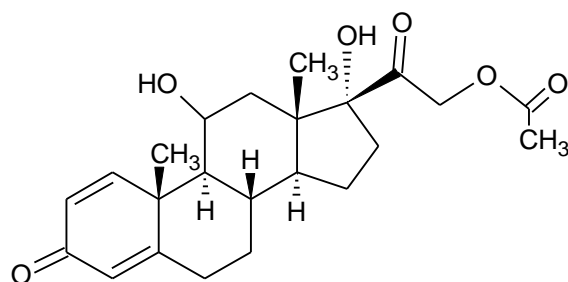
**Table 3: Regression analysis data and summary of validation parameters for the developed method**

Parameters	OFL	PRD	Isoabsorptive point
Wavelength range (nm)	243	243	236.80
Beer's law limit (µg/ml)	3-30	3-30	3-30
Regression equation (y=mx+c)	y = 0.0304x-0.0158	y = 0.0428x-0.0091	y = 0.0406x-0.0238
Slope(m)			
Intercept(c)	0.0304 0.0158	0.0428 0.0091	0.0406 0.0238
Correlation Coefficient (r <sup>2</sup> )	0.998	0.999	0.999
Repeatability (n=6) (%R.S.D)	0.44	0.20	0.26
Intraday (n=3) (%R.S.D)	0.42-0.85	0.30-0.63	0.32-0.81
Interday(n=3) (%R.S.D)	0.56-1.06	0.40-0.93	0.42-0.98
LOD (µg/ml)	0.72	0.71	0.94
LOQ (µg/ml)	2.2	2.1	2.8
Accuracy (n=3) (Mean % Recovery ± S.D)	99.66 ± 0.30	99.68 ± 0.39	-
%Assay ± S.D.(n=5)	100.4 % ± 1.07	100.1 % ± 0.76	-

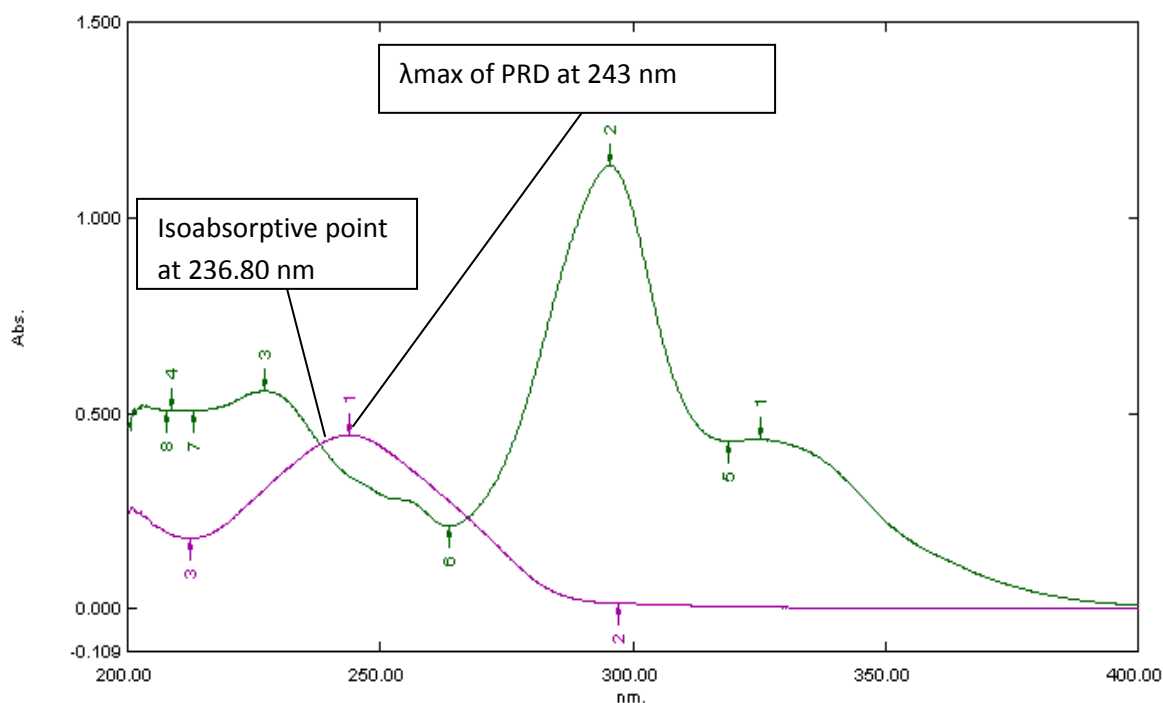
RSD=Relative standard deviation. LOD=Limit of detection. LOQ=Limit of quantification.  
SD=Standard deviation.



**Figure 1: Chemical structure of Ofloxacin (OFL)**



**Figure 2: Chemical structure of Prednisolone acetate (PRD)**



**Figure 3: Overlain spectrum OFL (10µg/ml) and PRD (10µg/ml) in methanol**

The validation parameters were studied at all the selected wavelengths for the developed method. All the validation parameters were found to be within acceptable limits. The % recoveries were found to be in the range of 99.44-99.88% for OFL and 99.43-100.03 % for PRD (Table 1). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the % RSD which indicates good method precision (Table 3). The Limit of detection 0.72 µg/ml at 243 nm for OFL, 0.71 µg/ml at 243 nm for PRD and 0.94 µg/ml at isoabsorptive point (236.80 nm). Limit of quantification for OFL and PRD was found at 243 nm were 2.2 µg/ml and 2.1 µg/ml respectively and at isoabsorptive point, LOQ was 2.8 µg/ml (Table 3). The proposed spectrophotometric method was successfully applied to OFL and PRD in binary mixture (Ophthalmic Formulation). OFL and



PRD content in marketed eye drops were found to be 100.4% and 100.1 % respectively (Table 2).

## CONCLUSION

The Q – absorbance ratio method was developed for simultaneous determination of OFL and PRD in binary mixture. Method was found to be precise and accurate as can be reflected from validation parameters data. Developed method was efficiently applied for determination of OFL and PRD in pharmaceutical formulation and there for method can be extended for the analysis of formulation.

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