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Research Article

HPLC-UV METHOD FOR SIMULTANEOUS DETERMINATION OF OFLOXACIN AND PREDNISOLONE ACETATE IN BULK AND FORMULATIONS

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Abstract

A simple, specific and stability indicating liquid chromatographic method was developed and validated for the simultaneous determination of ofloxacin and prednisolone acetate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 5 min using a C_{18} column (150 mmx4.6 mm i.d, 3µ particle size) by isocratic elution. The mobile phase consisting of a mixture of 0.01M phosphate buffer (pH 3.0) and acetonitrile (40:60, v/v) was used. Column effluents were monitored at 240 nm at a flow rate of 1ml/min. Retention times of ofloxacin and prednisolone acetate were 2.7 and 4.6 min respectively. The linearity of ofloxacin and prednisolone acetate was in the range of 1.5-9 µg/ml and 5-30 µg/ml respectively. Precision of the method and instrument precision was evaluated and relative standard deviation values were within the limits. Recoveries were between 98-101% for both ofloxacin and prednisolone acetate. Developed method was economical in terms of the time taken and amount of solvent consumed for each analysis. The method was validated and successfully applied to the simultaneous determination of ofloxacin and prednisolone acetate in bulk and pharmaceutical formulations.

Key Words: Simultaneous determination, HPLC, Isocratic elution, Validation.

Introduction

Ofloxacin (OFN) is a second generation fluoroquinolone, broad spectrum antibiotic used in bacterial infections. It is chemically (RS) -7 –fluoro 2 –methyl -6 – (4 –methylpiperazin -1 –yl) -10 –oxo -1 –azatricyclo [7.3.1.0] trideca -5 (13), 6,8,11 –tetraene -11 –carboxylic acid. Prednisolone acetate (PA) is a corticosteroid primarily with major glucocorticoid activity and low mineralocorticoid activity which is widely used in ocular inflammatory diseases to reduce swelling, itching and redness. Its chemical name is (11β) -11, 17, 21-trihydroxy pregna-1, 4 –diene -3, 20 –dione 21-acetate[1]. Combination of antibiotic with steroid is useful to resolve both infection and inflammation. The

combination can also be used for post operative inflammation and any other ocular inflammation associated with infection. Prednisolone in combination with ofloxacin is used in several anti-infective eye preparations to treat acute and sub acute conjunctivitis caused by susceptible strains of the following aerobic gram positive and negative bacteria such as S. aureus, S. epidermidis, S. pneumonia and haemophilus influenza[2].

In the literature, methods were reported for the estimation of PA by spectrophotometry[3], gas chromatography[4]. Few methods were also reported for the simultaneous determination of PA with other corticosteroids[5-8], salbutamol[9] and antibiotics[10]. Similarly methods are available for the estimation of OFN by spectrophotometry and spectrofluorimetry[11] and HPLC[12]. Simultaneous determination of ofloxacin with other drugs such as tetrazoline hydrochloride[13], cefexime[14] was also reported. According to our knowledge no HPLC method is reported for simultaneous determination of OFN and PA in the literature. So an attempt was made to develop a HPLC method for the simultaneous estimation of these drugs available as eye drops.

The purpose of the present study was to develop a simple, sensitive and specific HPLC method for determination of OFN and PA in bulk and pharmaceutical formulations simultaneously. The developed method has been validated[15,16] to determine its suitability for its intended use by parameters such as specificity, linearity, limit of detection and quantification, precision, accuracy by recovery studies and system suitability. The validated method was applied to the commercially available pharmaceutical formulations containing both the drugs.

Materials and Methods

Materials

OFN and PA were obtained as gift samples from Ajanta pharmaceuticals Ltd, Mumbai. HPLC grade acetonitrile was purchased from SD fine chemicals, India. Triple distilled water was used during the study. The pharmaceutical formulations containing 0.3mg/ml of OFN and 1mg/ml PA (OCEPRED eye drops, Sun Pharma Ltd, India.) was purchased from local market.

Instrumentation

A high performance liquid chromatograph (Shimadzu-10 AT VP) equipped with two pumps (Model-10AT VP) and Shimadzu UV-Visible detector (SPD-10AT VP), ultrasonic bath **(**Spincotech Pvt. Ltd, India).

Chromatographic conditions

For chromatographic analysis, YMC ODS C_{18} column (150 mmx4.6 mm i.d, 3µ particle size) was used. Separation was carried out by isocratic elution. The solvent system was a mixture of 0.01M phosphate buffer (pH 3.0) and

acetonitrile (ACN) in the ratio of 40:60, v/v. It was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 30 min before passing through the instrument. The injection volume was 20 µl and the flow rate was 1ml/min. UV detection was carried out at 240 nm. Chromatographic separations were carried out at room temperature (25-30°C).

Preparation of solutions

Weighed and transferred 7.5 mg of OFN and 25 mg of PA in 25 ml volumetric flask and made the solution with the mobile phase to obtain a concentration of 300 µg/ml and 1000 µg/ml of OFN and PA respectively. Prepared the working standard solutions by suitable dilution of the stock with the mobile phase.

Prepared the sample solution by diluting 5 ml of the ophthalmic solution to 25 ml to get a concentration of 150 µg/ml and 500 µg/ml of OFN and PA respectively. From this 0.5ml was taken and diluted to 10 ml to get a concentration of 7.5 µg/ml and 25 µg/ml of OFN and PA.

Method validation

The developed analytical method was validated as per ICH and USP guidelines for the parameters like linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision, specificity, accuracy, robustness, and system suitability.

Linearity: Six working standard solutions of each analyte in the concentration of 1.5-9 µg/ml for OFN and 5-30 µg/ml for PA were prepared in triplicate and injected. Calibration graphs were plotted between concentration and mean peak area.

Limits of detection and Quantification: According to ICH, limit of detection (LOD) is the smallest level of analyte that gives measurable response that can be detected and limit of quantification (LOQ) is the smallest concentration of analyte that can be detected with acceptable accuracy and precision. LOD and LOQ are calculated from the formulae $3.3\sigma/s$ and $10\sigma/s$ respectively. Where σ is the standard deviation of y-intercepts of the regression line and s is the slope of the calibration curve.

Precision: The precision was determined in terms of both intra and inter-day precision and by different analysts. For intra-day precision three distinct concentrations of OFN and PA in the linearity range was prepared in triplicate and was analyzed on the same day. For inter-day precision the same concentrations were analyzed on three consecutive days and RSD values were calculated.

Instrument precision was analyzed by injection repeatability. This was examined by analyzing six injections of the mixture containing 7.5 and 25 µg/ml of OFN and PA, respectively. RSD values were calculated from the peak areas and retention times (RT) of OFN and PA.

Accuracy: It was determined by the addition of appropriate amounts of OFN and PA to a sample solution of fixed concentration and comparing calculated and measured concentrations. A sample solution containing OFN and PA (0.15 mg/ml and 0.5 mg/ml, respectively) was prepared by dilution of 5 ml of the ophthalmic solution to 10 ml in volumetric flask, and made up to the mark with the mobile phase. Samples (0.2 ml) of the filtered solution was taken in 10 ml volumetric flasks containing 0.05, 0.1, and 0.15 ml of OFN and PA standard solution and analyzed.

Specificity: The chief excipient present in the eye drops is benzalkonium chloride which is used as preservative. Sample solution containing benzalkonium chloride was injected into the system and chromatogram was recorded.

Robustness: Robustness was evaluated by deliberately varying method parameters such as detection wavelength and flow rate. Detection wavelength was changed from 240 nm to 240±2 nm and flow rate was changed from 1ml/min to 1±0.1ml/min. Effect of these changed parameters was studied by injecting the sample in to the system.

System suitability: System suitability was established. Parameters including retention factor, asymmetry factor / tailing factor, resolution and plate number were used to determine system suitability.

Assay of the marketed formulation:

Content of OFN and PA in pharmaceutical formulations was determined by the developed method. Sample concentration was determined by carrying out six separate determinations and each series was injected in triplicate

Results and Discussion

Mobile phase optimization:

Chromatographic conditions were set to develop a HPLC method for estimation of OFN and PA simultaneously with analysis time < 5min, and good resolution (R_S>2). Various compositions of mobile phases like methanol: buffer and ACN: buffer in different ratios were tried. But with 0.01M phosphate buffer (pH 3.0) and ACN in 40:60 v/v, at a flow of 1ml/min symmetrical peaks with good resolution were obtained. The detection wavelength was set at 240 nm where good detector response was obtained for these drugs. The RT was 2.7 and 4.6 min for OFN and PA respectively (Figure 1).

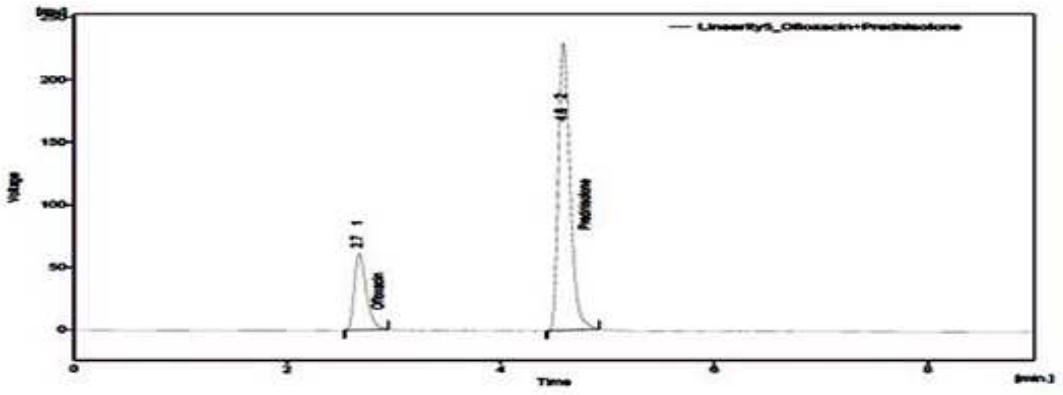

Figure 1: Typical chromatogram for the standard solution of Ofloxacin and Prednisolone acetate.

Validation:

Calibration graphs were constructed between the peak areas versus their corresponding concentrations. Good linearity was obtained in the concentration of 1.5-9 µg/ml and 5-30 µg/ml for OFN and PA and the results are shown in (Table 1). The precision of the method and instrument precision was evaluated and relative standard deviation (RSD) values were calculated. Low RSD values indicated satisfactory precision for both the drugs. The results are shown in (Table 2). Good recoveries were obtained and were between 98-101% for both OFN and PA; the results are given in (Table 3). Developed method was robust when the detection wavelength and flow rate was changed from 240 nm to 240±2 nm and 1 ml/min to 1±0.1 ml/min. There was no considerable change in the peak areas and RT. Using 0.9 ml/min flow rate, the RT for OFN and PA were 2.81 and 4.84 min respectively and with 1.1 ml/min flow rate, RT for OFN and PA were 2.45 and 4.37 min, respectively without affecting the resolution of the drugs. When detection wavelength was changed to 240±2 nm, the RT for OFN and PA were not changed from the normal. LOD and LOQ were calculated from the calibration curve. For OFN it was 0.032 and 0.098 µg/ml and for PA 0.096 and 0.29 µg/ml respectively. System suitability parameters are shown in (Table 4).

Table-1: Linearity by Regression Analysis (n=6).

Analyte	R^2	Slope	Conc. range(µg/ml)
OFN	0.9994	4.1475	1.5-9
PA	0.9994	6.6433	5-30

Table 2: Precision Expressed as %RSD

Parameters	OFN	PA
Intra-day precision	0.5-0.75	0.38-0.53
Inter-day precision	1.38-1.9	0.58-1.12
Analyst precision	0.45	0.63
Injection repeatability for RT	0.69	0.5
Injection repeatability for peak area	0.91	0.77

Table-3: Recovery Studies (n=6).

Analyte	Conc. (µg/ml)	Amount recovered (µg/ml)	% Recovery	% RSD
	4.5	4.51	100.32	0.57
OFN	6	6	100.02	1.32
	7.5	7.48	99.78	1.15
	15	15.1	100.68	0.91
PA	20	20.18	100.89	0.57
	25	24.95	99.81	0.24

Table-4: System Suitability Parameters (n=6).

Parameters	OFN	PA
Retention time	2.7	4.6
Asymmetry factor	1.5	1.48
Resolution	-	9.23
Number of plates	2609	8094
LOD (µg/ml)	0.032	0.096
LOQ (µg/ml)	0.098	0.292

Assay of the marketed formulation

The assay value of the marketed formulation was determined and RSD values were calculated. The results are given in (Table 5). In the chromatogram of the sample no interference was observed from the excipients, this indicates the specificity of the method (Fig. 2).

Table-5: Assay of Eye Drops (n=6).

Analyte	Label claim (mg/ml)	Amt. found (mg/ml)	Mean % Recovery	% RSD
OFN	0.3	0.3	100.18	1.15
PA	1	1	100.1	0.1

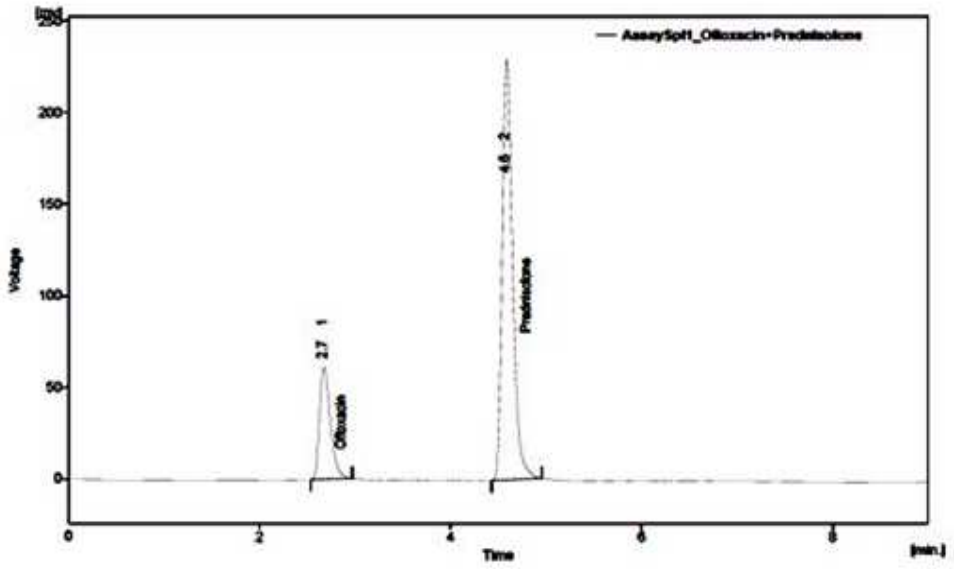

Figure 2: Typical chromatogram for the sample solution of Ofloxacin and Prednisolone acetate

Proposed method was found to be simple, sensitive, accurate and precise. With the optimized analytical conditions a good resolution was obtained within short time. The RSD for all parameters was well within the limits, which indicates the suitability of method and assay results obtained by this method is in good agreement with the labelled amount. Thus the developed method can be proposed for the analysis of OFN and PA in laboratories and for quality control purposes.

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