



HPLC-UV Method for Simultaneous Determination of Moxifloxacin and Prednisolone Acetate in Eye Drops

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A simple, sensitive liquid chromatographic method was developed and validated for the simultaneous estimation of moxifloxacin and prednisolone acetate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 10 min using a C₁₈ column (250 mm × 4.6 mm i.d, 5μ particle size) by isocratic elution. The mobile phase consisting of a mixture of mixed phosphate buffer (pH 6.8) and acetonitrile (50:50 v/v) was used. Column effluents were monitored at 265 nm at a flow rate of 1 mL/min. Retention times of moxifloxacin and prednisolone acetate were 3 and 4.2 min, respectively. The linearity of moxifloxacin and prednisolone acetate was in the range of 2-12 and 4-24 μg/mL respectively. 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 moxifloxacin and prednisolone acetate in bulk and pharmaceutical formulations.

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

INTRODUCTION

Moxifloxacin is a fourth generation fluoroquinolone antibiotic used in bacterial infections. It is chemically 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo(4.3.0)non-8-yl]-6-fluoro-8-methoxy-4-oxo-3-quinoline carboxylic acid. Moxifloxacin inhibits topoisomerase II and IV is an essential enzyme involved replication, transcription and repair of bacterial DNA^{1,2}. Prednisolone acetate is a corticosteroid with predominant glucocorticoid activity and low mineralocorticoid activity which is widely used in ocular inflammatory diseases to reduce swelling, itching and redness that affects the eye. Glucocorticoids inhibit the edema, fibrin deposition, capillary dialation and phagocytic migration of the acute inflammatory response as well as capillary proliferation, deposition of collagen and scar formation. Its chemical name is (11β)-11,17,21-trihydroxy pregna-1,4-diene-3,20-dione-21-acetate. Often combination of antibiotic with steroid exerts efficacy in resolving 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 moxifloxacin 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*.

In the literature, there are methods described for the individual estimation of fluoroquinolones and prednisolone in aqueous samples and biological fluids by liquid chromatography³, liquid-chromatography-fluorescence detection⁴, high performance thin layer chromatography⁵, spectrophotometry⁶ and by precipitation reagents⁷. A few methods have also been described for the simultaneous determination of moxifloxacin⁸ and prednisolone⁹ with other drugs such as ofloxacin, dexamethasone and hydrocortisone. But simultaneous determination of moxifloxacin and prednisolone acetate has not been reported in the literature. So an attempt was made to develop a HPLC method for the estimation of these drugs available as eye drops. The purpose of the present study was to develop a simple, sensitive and economical HPLC method for simultaneous determination of moxifloxacin and prednisolone acetate in bulk and pharmaceutical formulations. The developed method has been validated^{10,11} by evaluation of the system suitability, specificity, linearity, limit of detection and quantification, precision, accuracy and recovery. The validated method was applied to the commercially available pharmaceutical formulations containing both the drugs.

EXPERIMENTAL

Prednisolone acetate and moxifloxacin 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 0.5 mg/mL of moxifloxacin and 1 mg/mL prednisolone acetate was purchased from local market.

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) was used during the study.

Chromatographic conditions: For chromatographic analysis, a Chromosil C₁₈ column (250 mm × 4.6 mm i.d, 5 μ particle size) was used. Separation was carried out by isocratic elution. The mobile phase consisting of a mixture of mixed phosphate buffer (pH 6.8) and acetonitrile (ACN) in the ratio of 50:50, v/v was used. Mobile phase was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 0.5 h before passing through the instrument. The injection volume was 20 μL and the flow rate was 1 mL/min. UV detection was carried out at 265 nm. Chromatographic separations were carried out at room temperature (25-30 °C).

Preparation of standard solution: Stock standard solutions of moxifloxacin and prednisolone acetate were prepared in the mobile phase at a concentration of 200 and 400 μg/mL. Working standard solutions were prepared by serial dilution of stock solutions with the mobile phase.

Preparation of sample solution: Sample solutions of moxifloxacin and prednisolone acetate were prepared at a concentration of 200 and 400 μg/mL by diluting 10 mL of the ophthalmic solution to 25 mL with the mobile phase. From this 0.5 mL was taken and diluted to 10 mL to get a concentration of 10 μg/mL and 20 μg/mL of moxifloxacin and prednisolone acetate respectively.

Method validation: The developed analytical method was validated as per ICH and USP guidelines for the parameters like linearity, 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 range of 2-12 μg/mL for moxifloxacin and 4-24 μg/mL for prednisolone acetate were prepared in triplicate and injected. Calibration curves were constructed by plotting concentration versus mean peak area.

Limit of detection and limit of quantification: According to ICH, limit of detection (LOD) is the lowest concentration of the analyte that can be detected and limit of quantification (LOQ) is the lowest 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 of the method was evaluated by intermediate precision which include intra-day and inter-day precision and precision by different analysts. For intra-day precision three different concentrations of moxifloxacin and prednisolone acetate in the linearity range were prepared in triplicate and was analyzed during 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

10 and 20 μg/mL of moxifloxacin and prednisolone acetate, respectively. RSD values were calculated from the peak areas and retention times of moxifloxacin and prednisolone acetate.

Accuracy: Accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amounts of moxifloxacin and prednisolone acetate to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution containing moxifloxacin and prednisolone acetate (0.2 and 0.4 mg/mL, respectively) was prepared by diluting 10 mL of the ophthalmic solution to 25 mL in a volumetric flask and make up the solution with the mobile phase. Samples (0.2 mL) of the filtered solution were transferred to 10 mL volumetric flasks containing 0.1, 0.2 and 0.3 mL of moxifloxacin and prednisolone acetate standard solution and analyzed.

Specificity: Specificity of an analytical method can be defined as the ability of the method to measure accurately and specifically the analyte in presence of additional components such as matrix, impurities, degradation products and other related substances. The main 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 of the method was evaluated by deliberately varying method parameters such as detection wavelength and flow rate. Detection wavelength was changed from 265 nm to 265 ± 2 nm and flow rate was changed from 1 mL/min to 1 ± 0.1 mL/min. Effect of these changed parameters was studied by injecting the sample in to the system.

System suitability: System suitability was established in order to determine the adequate resolution and reproducibility of the proposed method. Suitability parameters including retention factor, resolution, asymmetry factor, plate number were investigated.

Assay of the marketed formulation: The developed method was applied to the simultaneous determination of moxifloxacin and prednisolone acetate in pharmaceutical formulations. Sample was analyzed by performing six independent determinations and each series was injected in triplicate.

RESULTS AND DISCUSSION

Mobile phase optimization: Chromatographic parameters were optimized to develop a HPLC method for simultaneous determination of moxifloxacin and prednisolone acetate with short analysis time (< 5 min) and acceptable resolution ($RS > 2$). Various compositions of mobile phases like methanol: buffer and acetonitrile: buffer in different ratios were tried. But with mixed phosphate buffer (pH 6.8) and acetonitrile in the ratio of 50:50 at a flow rate of 1 mL/min, symmetrical peaks with good resolution were obtained. Chromatogram for the mobile phase (blank chromatogram) is shown in Fig. 1 and no interference was observed with the drug peaks. The optimum wavelength for detection was set at 265 nm at which better detector response for both drugs was obtained. The retention times were 3 and 4.2 min for moxifloxacin and prednisolone acetate respectively (Fig. 2).

Validation: Calibration graphs were constructed between the peak areas *versus* their corresponding concentrations. Good linearity was obtained in the range of 2-12 and 4-24 μg/mL

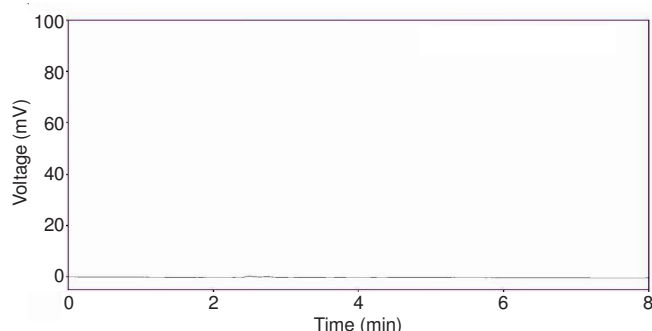


Fig. 1. Chromatogram for blank

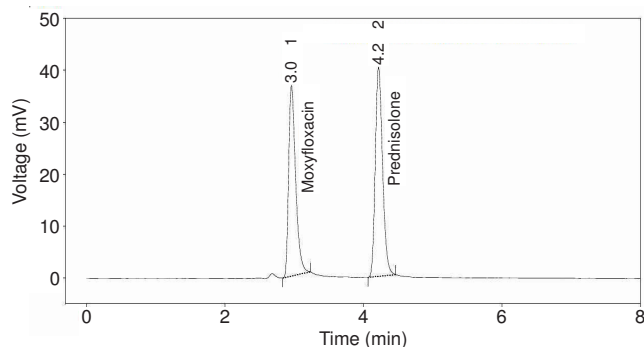


Fig. 2. Typical chromatogram for the standard solution of moxifloxacin and prednisolone acetate

for moxifloxacin and prednisolone acetate. The results are shown in Table-1. LOD and LOQ were determined from the slope and standard deviation of y-intercepts of the regression line of the calibration curve. For moxifloxacin it was found to be 0.030 and 0.092 $\mu\text{g/mL}$ and for prednisolone acetate 0.073 and 0.22 $\mu\text{g/mL}$ respectively. The precision of the method and instrument precision was evaluated and relative standard deviation (RSD) values were calculated. The RSD values for moxifloxacin and prednisolone acetate showed that the precision of the method was satisfactory. The results are shown in Table-2. The accuracy of the method was determined by recovery studies. The recoveries were close to 100 % for moxifloxacin and prednisolone acetate (Table-3). Developed method was found to be robust when the detection wavelength and flow rate was changed from 265 nm to 265 ± 2 nm and 1 mL/min to 1 ± 0.1 mL/min. There was no considerable change in the peak areas and retention times. Using 0.9 mL/min flow rate, the retention time for moxifloxacin and prednisolone acetate were found to be 3.32 and 4.45 min, respectively and with 1.1 mL/min flow rate, retention times for moxifloxacin and prednisolone acetate were found to be 2.91 and 4.07 min, respectively without affecting the resolution of the drug components. When detection wavelength was changed to 265 ± 2 nm, the retention time for moxifloxacin and prednisolone acetate were not changed from the normal. System suitability parameters are shown in Table-4.

TABLE-1
LINEARITY BY REGRESSION ANALYSIS (n=6)

Substance	R ²	Slope	Conc. range ($\mu\text{g/mL}$)
Moxifloxacin	0.9998	43.79	2-12
Prednisolone acetate	0.9997	25.17	4-24

'n' is number of determinations.

TABLE-2
PRECISION EXPRESSED AS
% RELATIVE STANDARD DEVIATION

Parameters	Moxifloxacin	Prednisolone acetate
Intra-day precision	0.39	0.59
Inter-day precision	1.35	0.88
Analyst precision	0.14	0.28
Injection repeatability for t_R	0.77	0.51
Injection repeatability for peak area	0.90	0.81

TABLE-3
RECOVERY STUDIES (n = 6)

Drug	Concentration ($\mu\text{g/mL}$)	Amount recovered ($\mu\text{g/mL}$)	Recovery (%)	% RSD
Moxifloxacin	6	5.95	99.09	1.03
	8	8.05	100.58	0.72
	10	10	100.04	0.44
Prednisolone acetate	12	11.92	99.33	1.03
	16	15.96	99.75	0.83
	20	19.92	99.6	1.14

'n' is number of determinations and RSD is relative standard deviation

TABLE-4
SYSTEM SUITABILITY PARAMETERS (n = 6)

Parameters	Moxifloxacin	Prednisolone acetate
Retention time (t_R)	3	4.2
Asymmetry factor	1.6	1.3
Resolution	-	6.75
Number of plates	4266	7693

'n' is number of determinations.

Assay of the marketed formulation: According to ICH in case of assay, demonstration of specificity requires that the procedure is unaffected by the presence of impurities or excipients. The assay value of the marketed formulation was found to be within the limits. The low RSD value indicated suitability of this method for routine analysis of moxifloxacin and prednisolone acetate in pharmaceutical dosage forms. Chromatogram of the sample (Fig. 3) shows that there was no interference from the excipients present in the formulation; this indicates the specificity of the method. The results are shown in Table-5.

Conclusion

The method described in this paper for the simultaneous estimation of moxifloxacin and prednisolone acetate was found

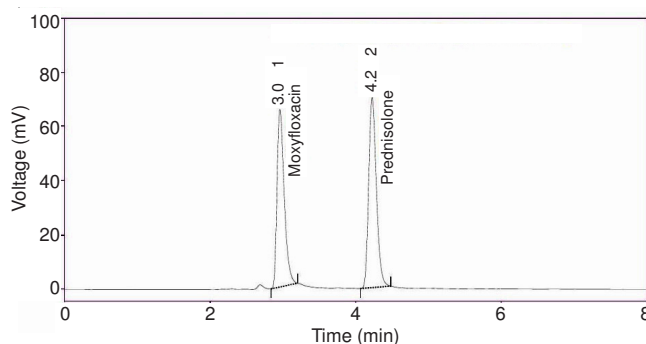


Fig. 3. Chromatogram for the sample solution of moxifloxacin and prednisolone acetate

TABLE-5
ASSAY OF EYE DROPS (n = 6)

Drug	Label claim (mg/mL)	Amt. Found (mg/mL)	Mean recovery (%)	% RSD
Moxifloxacin	0.5	0.501	100.3	0.13
Prednisolone acetate	1	0.99	99.6	0.268

'n' is number of determinations and RSD is relative standard deviation

to be simple, sensitive, accurate, precise, rapid, robust and economical. The analytical conditions and the solvent system developed provided good resolution within a short analysis time. The RSD for all parameters was found to be within the limits, which indicates the validity of method and assay results obtained by this method are in fair agreement. Thus the developed method can be proposed for routine analysis of moxifloxacin and prednisolone acetate in laboratories and for quality control purposes.

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