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Research Article

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CHLORAMPHENICOL 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 chloramphenicol and prednisolone acetate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 5 min using a C_{18} column (250 mmx4.6 mm i.d, 5µ particle size) by isocratic elution. The mobile phase consisting of a mixture of mixed phosphate buffer (pH 5.0) and acetonitrile (40:60, v/v) was used. Column effluents were monitored at 259 nm at a flow rate of 1ml/min. Retention times of chloramphenicol and dexamethasone sodium phosphate were 2.58 and 3.58 min respectively. The linearity of chloramphenicol and prednisolone acetate was in the range of 2-12 µg/ml and 5-30 µg/ml respectively. Chloramphenicol and prednisolone acetate were subjected to forced degradation by acid, alkali, chemical oxidation and heat. 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 chloramphenicol and dexamethasone sodium phosphate in bulk and pharmaceutical formulations.

Keywords: Simultaneous determination, HPLC, Isocratic elution, Validation, Forced degradation

INTRODUCTION

Chloramphenicol (CPL) is a broad spectrum antibiotic used in bacterial infections. It is chemically 2, 2-dichloro-[1, 3-dihydroxy-1-(4-nitrophenyl) propan-2-yl] acetamide. 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 chloramphenicol 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, there are methods described for the estimation of CPL by spectrophotometry[3], high performance liquid chromatography[4] (HPLC), gas chromatography[5]. A few methods have also been described for the simultaneous determination of CPL with other drugs such as dexamethasone[6]. Similarly methods were reported for the determination of PA by spectrophotometry[7], gas chromatography[8]. A few methods were also given for the simultaneous determination of PA with other corticosteroids[9-12], salbutamol[13] and antibiotics[14, 15]. According to our knowledge no stability indicating HPLC method is reported for simultaneous determination of CPL and PA in the literature. So an attempt was made to develop a stability indicating 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 CPL and PA in bulk and pharmaceutical formulations simultaneously. The developed method has been validated[16, 17] to determine its suitability for its intended use by parameters such as specificity, linearity, limit of detection and quantification, precision, accuracy and system suitability.

MATERIALS AND METHODS

Materials

CPL 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 0.2% w/v of CPL and 0.5% w/v of PA (CHLORAMSONE eye drops, Ranbaxy 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, a Hypersil C_{18} column (250 mmx4.6 mm i.d, 5µ particle size) was used. Separation was carried out by isocratic elution. The solvent system was a mixture of mixed phosphate buffer (pH 5.0) 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 20µl and the flow rate was 1ml/min. UV detection was carried out at 259 nm. Chromatographic separations were carried out at room temperature (25-30˚C).

Preparation of solutions

Weighed and transferred 10 mg of CPL and 25 mg of PA in 25 ml volumetric flask and made the solution with the mobile phase to obtain a concentration of 400 µg/ml and 1000 µg/ml of CPL and PA respectively. Prepared the working standards by suitable dilutions of the stock with the mobile phase.

Prepared the sample solution by diluting 10ml of the ophthalmic solution to 25 ml to get a concentration of 200 µg/ml and 500 µg/ml of CPL and PA respectively. From this 0.5ml was taken and diluted to 10 ml to get a concentration of 10 µg/ml and 25 µg/ml of CPL and PA.

Method validation

The developed method was validated for "Linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision, specificity, accuracy, robustness, and system suitability" according to ICH and USP guidelines.

Linearity

Six working standard solutions of each analyte in the concentration of 2-12 µg/ml for CPL 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 3.3σ/s and 10σ/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 CPL 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 analysing six injections of the mixture containing 10 and 25 µg/ml of CPL and PA, respectively. RSD values were calculated from the peak areas and RT of CPL and PA.

Accuracy

It was determined by the addition of appropriate amounts of CPL and PA to a sample solution of fixed concentration and comparing calculated and measured concentrations. A sample solution containing CPL and PA (40 µg/ml and 100 µg/ml, respectively) was prepared by dilution of 5 ml of the ophthalmic solution to 25 ml in volumetric flask, and made up to the mark with the mobile phase. Samples (1.0ml) of the filtered solution was taken in 10 ml volumetric flasks containing 0.5, 1, and 1.5 ml of CPL 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 259 nm to 259±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.

Forced degradation study

To evaluate the stability of the proposed method forced degradation study was carried out using stress conditions such as exposure to acid, alkali, chemical oxidant and heat. The interference caused by the degradation products was investigated. In basic media forced degradation study was carried out by taking separately 5 ml solutions (stock) of CPL and PA in 25 ml volumetric flasks; to it 5 ml of 0.1 N NaOH was added and all the flasks were kept at room temperature for 24 hrs. Solutions were neutralized with acid using pH meter and suitably diluted to a final concentration of 4 µg/ml of CPL and 10 µg/ml of PA. In the same way degradation was carried out in acidic medium using 0.1 N HCl. Oxidative stress degradation was carried out similarly using 3% hydrogen peroxide solution. All the mixtures were kept at room temperature for 24 hrs. To study heat degradation, drug solutions were exposed to heat in an oven at 80 °C for 24 h. Solutions were diluted to obtain final concentration of 4 µg/ml of CPL and 10 µg/ml of PA with the mobile phase. All the solutions were injected in to the system and chromatograms were recorded.

Assay of the marketed formulation

Content of CPL and PA in pharmaceutical formulations was determined by the developed method. 20 µl of the diluted sample solution was injected into the system and chromatograms were recorded. Sample concentration was determined from the calibration curve.

RESULTS AND DISCUSSION

Mobile phase optimization

Chromatographic conditions were set to develop a HPLC method for estimation of DSP and CPL simultaneously with analysis time of less than 5 min, and good resolution. Various compositions of mobile phases like methanol: buffer and ACN: buffer in different ratios were tried. But with 0.01M phosphate buffer (pH 5.0) and ACN in 40:60 v/v and at a flow of 1ml/min, symmetrical peaks with good resolution were obtained. The detection wavelength was set at 259 nm where good detector response was obtained for these drugs. The retention times (RT) were 2.58 and 3.58 min for CPL and PA respectively (Fig. 1).

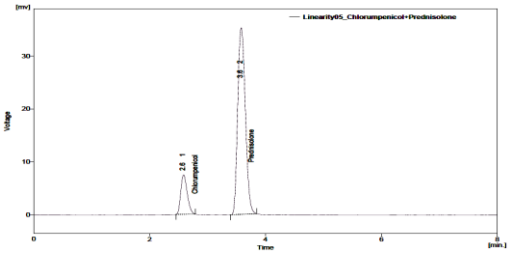

Fig. 1:Typical chromatogram for the standard solution of CPL and PA

Validation

Calibration graphs were constructed between the peak areas versus their corresponding concentrations. Good linearity was obtained in the concentration of 2-12 µg/ml and 5-30 µg/ml for CPL 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 found to be between 99.5-101% for both CPL and PA; the results are given in the Table 3. Developed method was robust when the detection wavelength and flow rate was changed from 259 nm to 259±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 CPL and PA were 2.72 and 3.69 min respectively and with 1.1 ml/min flow rate, RT for CPL and PA were found to be 2.39 and 3.35 min, respectively without affecting the resolution of the drugs. When detection wavelength was changed from 259 to 259±2 nm, the RT for CPL and PA were not changed from the normal. LOD and LOQ were determined from the calibration curve. For CPL it was 0.126 and 0.382 µg/ml and for PA 0.147 and 0.445 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)
CPL	0.9992	15.01	2-12
PA	0.9987	41.25	5-30

Table 2 Precision expressed as %RSD

Parameters	CPL	PA
Intra-day precision	0.3-1.5	0.23-0.53
Inter-day precision	0.8-1.4	0.21-0.48
Analyst precision	0.21	0.15
Injection repeatability for t_R	0.52	0.64
Injection repeatability for peak area	1.31	0.62

RSD is Relative Standard Deviation

Table 3 Recovery studies (n=6)

Analyte	Concentration µg/ml	Amount recovered µg/ml	% Recovery	% RSD
CPL	6	5.97	99.92	1.8
	8	8.09	101.07	1.48
	10	10.09	100.9	1.8
PA	15	14.92	99.45	1.21
	20	19.93	99.65	2.04
	25	24.9	99.62	1.07

Table 4 System suitability parameters (n=6)

Parameters	CPL	PA
Retention time	2.58	3.58
Asymmetry factor	1.42	1.29
Resolution	-	4.28
Number of plates	3437	3156
LOD (µg/ml)	0.126	0.147
LOQ (µg/ml)	0.382	0.445

Forced degradation study

Forced degradation was performed by exposing the drugs to acid, alkali, hydrogen peroxide and heat. Formation of degradation products were confirmed by the presence of different peaks at various RT and also by the decrease in the peak area of analytes. During this study base degraded sample showed degradation peaks at RT 2.3, 2.4, 3.6, and 4.2 min for CPL and at 3.2 and 3.8 min for PA (Fig. 2 and 3). Similarly acid degraded sample showed degradation peaks at RT 2.2, 2.4, 2.9, 3.6, and 6.3 min for CPL and at 2.2, 2.4, 2.6, 2.9 min, and at 4.2 min for PA (Fig.4 and 5). The chromatograms of heat degraded sample showed degradation peaks at RT 2.4, 3.6, and 4.2 min for CPL and at 2.2, 2.4, and 2.6 min for PA (Fig. 6 and 7). Chemical oxidation peaks were observed at RT 3.6 for CPL and at 2.4 and 2.7 min for PA (Fig. 8 and 9). Good resolution between drug peaks and degradation peaks were observed. The degradation study results indicated that PA was highly susceptible to degradation by alkaline hydrolysis and percentage recovery was negligible (Table 6).

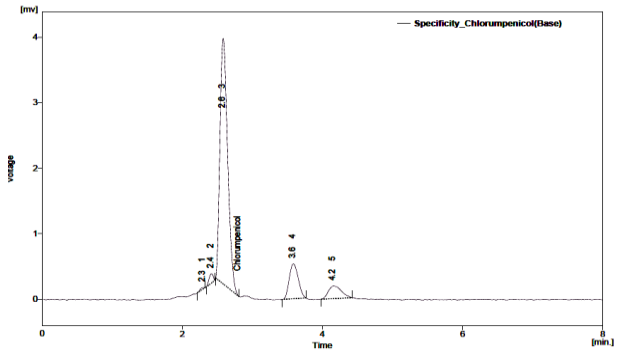

Fig. 2:Chromatogram of base treated CPL

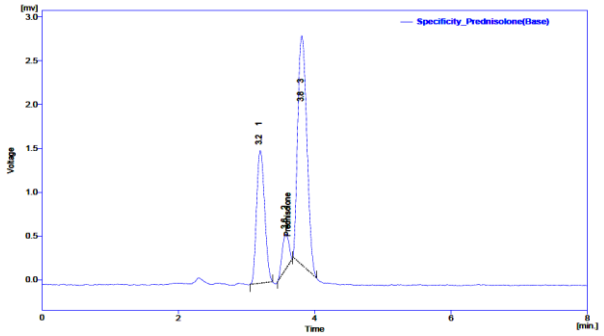

Fig. 3:Chromatogram of base treated PA

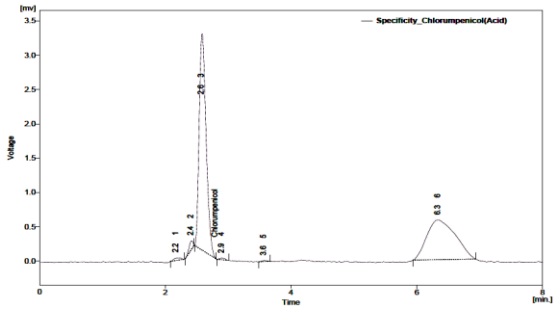

Fig. 4:Chromatogram of acid treated CPL

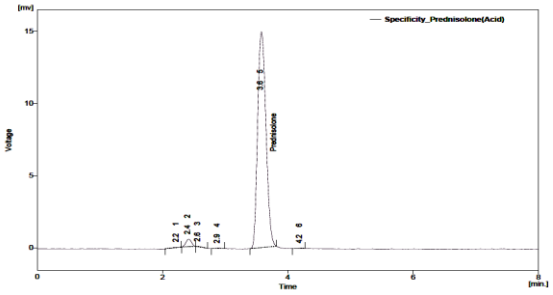

Fig. 5:Chromatogram of acid treated PA

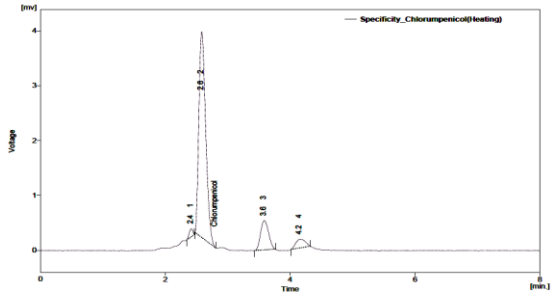

Fig. 6:Chromatogram of heat treated CPL

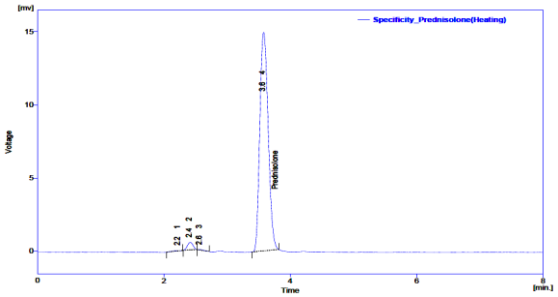

Fig. 7:Chromatogram of heat treated PA

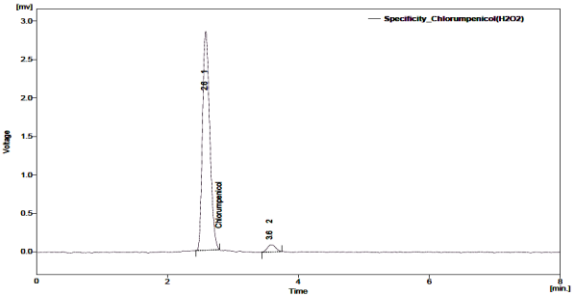

Fig. 8:Chromatogram of H_2O_2 treated CPL

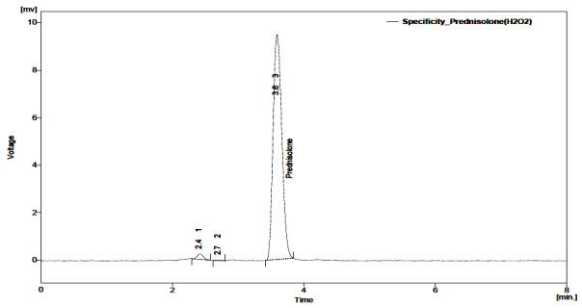

Fig. 9:Chromatogram of H_2O_2 treated PA

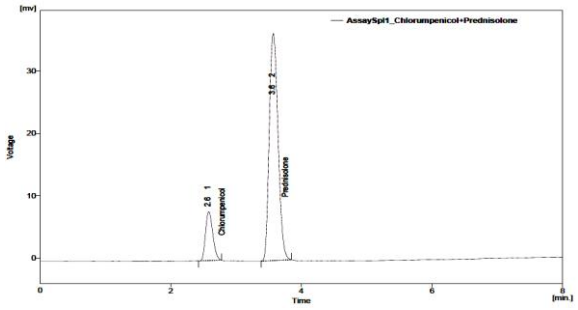

Fig. 10:Typical chromatogram for the sample solution of CPL and PA

Table 6 Forced degradation studies of CPL and PA

Condition	Time (hr)	% Recovery CPL	% Recovery PA	RT of degradation products CPL	RT of degradation products PA
Acid 0.1 N HCl	24	41.7	42.41	2.2, 2.4, 2.9, 3.6, 6.3	2.2, 2.4, 2.6, 2.9, 4.2
Base 0.1 NaOH	24	49.46	0.83	2.3, 2.4, 3.6, 4.2	3.2, 3.8
3% H2O2	24	38.03	26.91	3.6	2.4, 2.7
Heat	24	49.46	42.41	2.4, 3.6, 4.2	2.2, 2.4, 2.6

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. 10).

Table 5 Assay of eye drops (n=6)

Analyte	Label claim mg/ml	Amt. found mg/ml	Mean % Recovery	% RSD
CPL	0.2	0.199	99.6	0.97
PA	0.5	0.499	99.85	0.36

CONCLUSION

Proposed method was found to be specific, accurate, precise and rapid. 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 are in good agreement with the labeled amount. Thus the developed method can be proposed for the analysis of CPL and PA in laboratories and for quality control purposes.

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