Academic Sciences
International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DEXAMETHASONE SODIUM PHOSPHATE AND CHLORAMPHENICOL IN BULK AND FORMULATIONS

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Received: 22 May 2012, Revised and Accepted: 27 Jun 2012

ABSTRACT

A simple, specific and stability indicating liquid chromatographic method was developed and validated for the simultaneous determination of dexamethasone sodium phosphate (DSP) and chloramphenicol (CPL) 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 6.8) and acetonitrile (70:30, v/v) was used. Column effluents were monitored at 254 nm with a flow rate of 1ml/min. Retention times (RT) of dexamethasone sodium phosphate and chloramphenicol were 2.3 and 4.7 min respectively. The linearity of chloramphenicol and dexamethasone sodium phosphate was in the range of 5-30 µg/ml and 1-6 µg/ml respectively. Chloramphenicol and dexamethasone solutions 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 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

Dexamethasone sodium phosphate is a highly selective glucocorticoid which is widely used in ocular inflammatory diseases. Its chemical name is 9- fluoro-11b, 17, 21-trihydroxy-16α- methylpregna-1, 4- diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt[1]. Chloramphenicol 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. Dexamethasone 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, methods were reported for the estimation of CPL by spectrophotometry[3], high performance liquid chromatography (HPLC)[4], gas chromatography[5]. Similarly methods were reported for the determination of DSP by HPLC[6, 7], gas chromatography[8]. A few methods were also given for the simultaneous determination of Dexamethasone/Chloremphenicol with other drugs such as ciprofloxacin and ofloxacin[9-12]. Simultaneous determination of DSP and CPL has been reported in the literature[13, 14], but no stability indicating method with degradation studies is reported 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, specific and economical HPLC method for determination of DSP and CPL in bulk and pharmaceutical formulations simultaneously. The developed method has been validated[15, 16] to determine its suitability for 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

DSP and CPL 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 5mg/ml of CPL and 1mg/ml DSP (SONEXA-C eye drops, PSA chemicals & pharmaceuticals Ltd, India.) was purchased from local market.

Instrumentation

A high performance liquid chromatography (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 6.8) and acetonitrile (ACN) in the ratio of 70:30, 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 254 nm. Chromatographic separations were carried out at room temperature (25-30°C).

Preparation of solutions

Weighed and transferred 5 mg of DSP and 25 mg of CPL in 25 ml volumetric flask and made the solution with the mobile phase to obtain a concentration of 200 µg/ml and 1000 µg/ml of DSP and CPL respectively. Prepared the working standards by suitable dilutions 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 200 µg/ml and 1000 µg/ml of DSP and CPL respectively. From this 0.25ml was taken and diluted to 10 ml to get a concentration of 5 µg/ml and 25 µg/ml of DSP and CPL.

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 range of 5-30 µg/ml for CPL and 1-6 µg/ml for DSP were prepared in triplicate and injected. Calibration curves were plotted between concentration on X- axis and mean peak area/response on Y- axis.

Limits of detection and Quantification

According to ICH, limit of detection (LOD) is the smallest level of analyte that gives measurable response 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 DSP and CPL 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 5 and 25 µg/ml of DSP and CPL respectively. RSD values were calculated from the peak areas and RTs of DSP and CPL.

Accuracy

It was determined by the addition of appropriate amounts of DSP and CPL to a sample solution of fixed concentration and comparing calculated and measured concentrations. A sample solution containing DSP and CPL (0.2 and 1.0 mg/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 (0.1ml) of the filtered solution was taken in 10 ml volumetric flasks containing 0.05, 0.1, and 0.15 ml of DSP and CPL 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 254 nm to 254±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 5 ml solutions (stock) of DSP and CPL separately into two 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 h. Solutions were neutralized with acid using pH meter and suitably diluted to a final concentration of 1 µg/ml of DSP and 5 µg/ml of CPL. 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 h. To study heat degradation, drug solutions were exposed to heat in a oven at 80 °C for 24 h. Solutions were diluted to obtain final concentration of 1 µg/ml of DSP and 5 µg/ml of CPL and were injected in to the system and chromatograms were recorded.

Assay of the marketed formulation

Content of DSP and CPL 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 DSP and CPL simultaneously with analysis time< 5min, and good resolution. Various compositions of mobile phases like methanol: buffer and ACN: buffer in different ratios were tried. But with mixed phosphate buffer (pH 6.8) and ACN in 70:30 v/v, at a flow rate of 1ml/min symmetrical peaks with good resolution were obtained. The detection wavelength was set at 254 nm where good detector response was obtained for these drugs. The RTs were 2.3 and 4.7 min for DSP and CPL respectively (Fig.1).

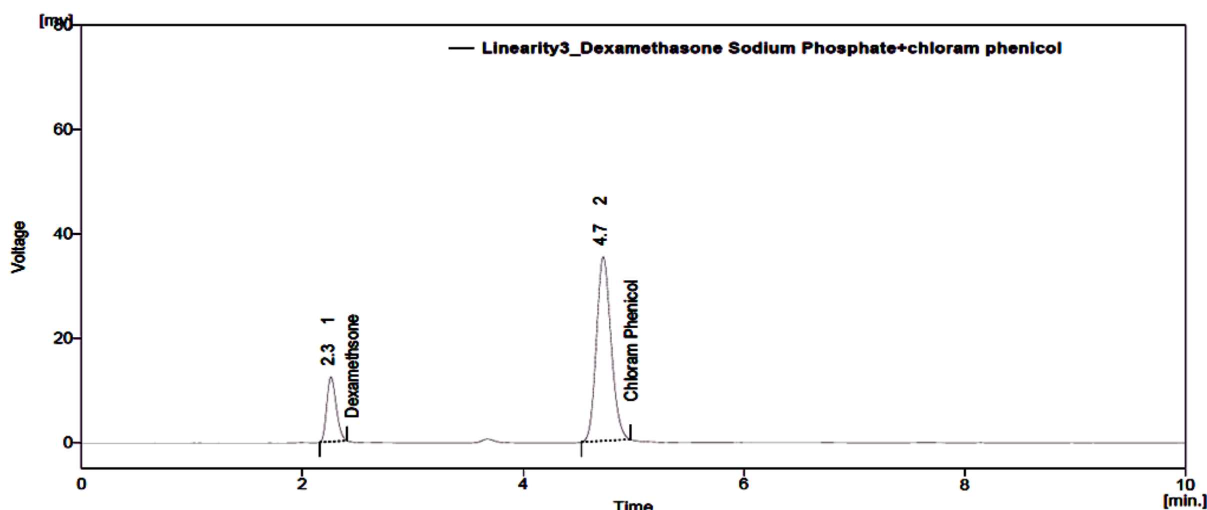

Fig. 1: Typical chromatogram for the standard solution of DSP and CPL

Table 1: Linearity by regression analysis (n=6)

Substance	R^2	Slope	Conc. range(µg/ml)
DSP	0.9989	24.05	1-6
CPL	0.9997	21.7	5-30

Validation

Calibration graphs were constructed between the peak areas versus their corresponding concentrations. Good linearity was obtained in the concentration of 1-6 µg/ml and 5-30 µg/ml for DSP and CPL 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 98-101% for both DSP and CPL; the results are given in the Table 3. Developed method was robust when the detection wavelength and flow rate was changed from 254 nm to 254±2 nm and 1ml/min to 1±0.1ml/min. There was no considerable change in the peak areas and RT. Using 0.9 ml/min flow rate, the RT for DSP and CPL were 2.49 and 4.92 min respectively and with 1.1 ml/min flow rate, RT for DSP and CPL were 2.06 and 4.53 min, respectively without affecting the resolution of the drugs. When detection wavelength was changed to 254±2 nm, the RT for DSP and CPL were not changed from the normal.

LOD and LOQ were determined from the calibration curve. For DSP it was 0.059 and 0.178 µg/ml and for CPL 0.157 and 0.477 µg/ml respectively. System suitability parameters are shown in Table 4.

Table 2: Precision expressed as %RSD

Parameters	DSP	CPL
Intra-day precision	0.5-1.95	0.79-1.5
Inter-day precision	0.5-2.05	1.56-1.97
Analyst precision	0.17	0.12
Injection repeatability for t_R	0.74	0.04
Injection repeatability for peak area	1.46	0.72

Table 3: Recovery studies (n=6)

Drug	Concentration µg/ml	Amount recovered µg/ml	% Recovery	% RSD*
DSP	3	3.01	100.33	1.75
	4	3.97	99.27	0.95
	5	5.01	100.16	1.31
CPL	15	14.9	99.31	0.40
	20	20.15	100.75	0.14
	25	24.93	99.73	0.73

*RSD is relative standard deviation

Table 4: System suitability parameters (n=6)

Parameters	DSP	CPL
Retention time	2.3	4.7
Asymmetry factor	1.45	1.3
Resolution	-	11.962
Number of plates	3037	5778
LOD (µg/ml)	0.059	0.157
LOQ (µg/ml)	0.178	0.477

Forced degradation study

Forced degradation was performed by exposing the drugs to acid, alkali, chemical oxidation 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. Base degraded sample showed degradation peaks at RTs 2.5, 2.9, 3.4, 3.8 and 4.7 min for DSP and at 1.9, 2.2, 2.4, 2.6, 2.9 and 4.3 min for CPL (Fig.2, 3). Similarly acid degraded sample showed degradation peaks at RT 2.5 and 3.8 min for DSP and at 2.2 and 2.6 min for CPL (Fig.4, 5). The chromatograms of heat degraded sample showed degradation peaks at RT 2.5 and 4.7 min for DSP and at 1.88, 2.2, 2.58, 2.94 and 4.28 min for CPL (Fig.6, 7). Chemical oxidation peaks were observed at RT 2.6 and 3.7 min for DSP and 2.4 and 2.5 min for CPL (Fig.8, 9). Good resolution between drug peaks and degradation peaks were observed. The degradation study results indicated that DSP was highly susceptible to degradation in the proposed conditions and percentage recoveries were small after degradation. Whereas CPL is considerably stable to acidic and oxidative stress and highly susceptible to degradation in alkaline media and by heat (Table 5).

Assay of the marketed formulation

According to ICH in the 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 determined and RSD values were calculated. The results are given in Table 6. Chromatogram of the sample shows that there were no additional peaks and hence no interference from the excipients presents in the formulation; this indicates the specificity of the method (Fig. 10).

Table 5: Forced degradation study of CPL and DSP

Condition	Time (h)	% Recovery		RT of degradation products	
		CPL	DSP	CPL	DSP
Acid 0.1 N HCl	24	77.1	19.44	2.2, 2.6	2.5, 3.8
Base 0.1 NaOH	24	5.91	23.17	1.9, 2.2, 2.4, 2.6, 2.9,4.3	2.5, 2.9, 3.4, 3.8, 4.7
3% H2O2	24	96.06	0	2.4, 2.5	2.4, 2.6, 3.7
Heat	24	5.91	30.08	1.9, 2.2, 2.6, 1.9, 4.3	2.5,2.7

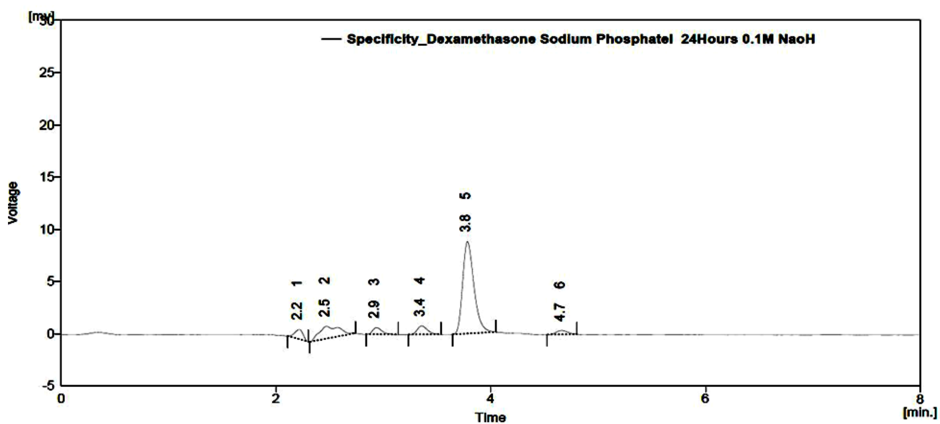

Fig. 2: Chromatogram of base treated DSP

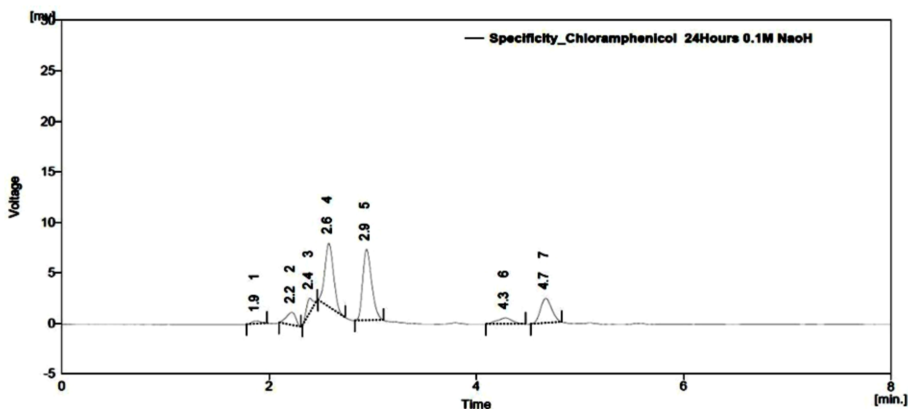

Fig. 3: Chromatogram of base treated CPL

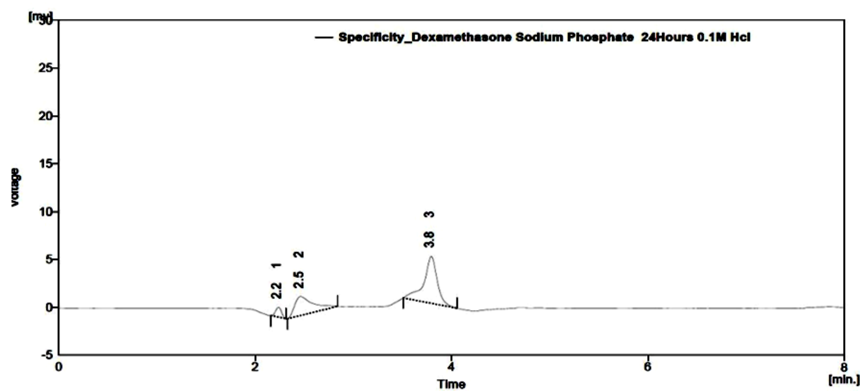

Fig. 4: Chromatogram of acid treated DSP

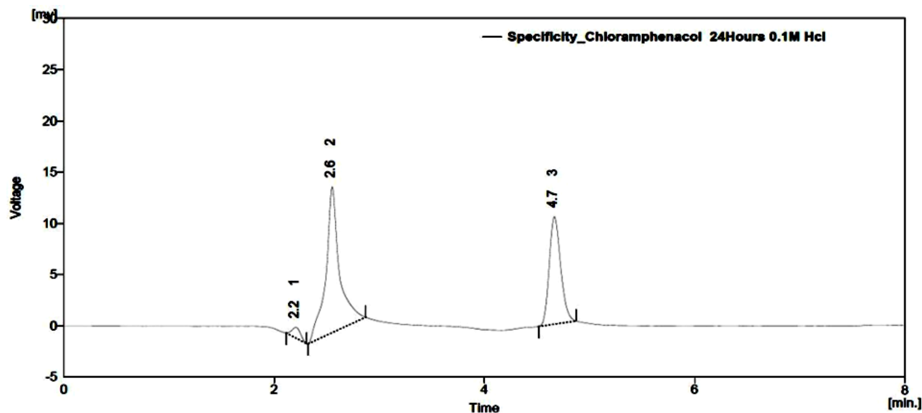

Fig. 5: Chromatogram of acid treated CPL

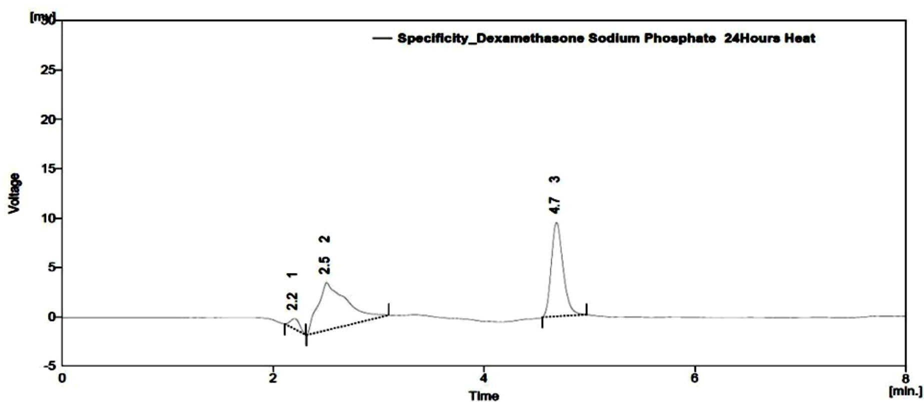

Fig. 6: Chromatogram of heat treated DSP

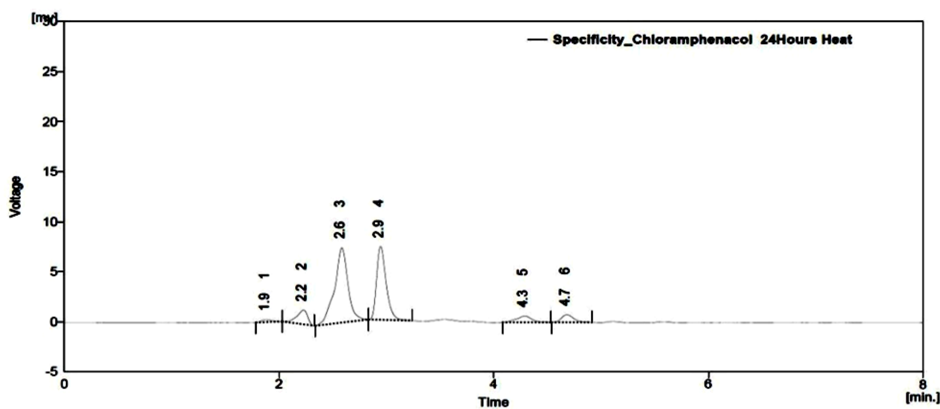

Fig. 7: Chromatogram of heat treated CPL

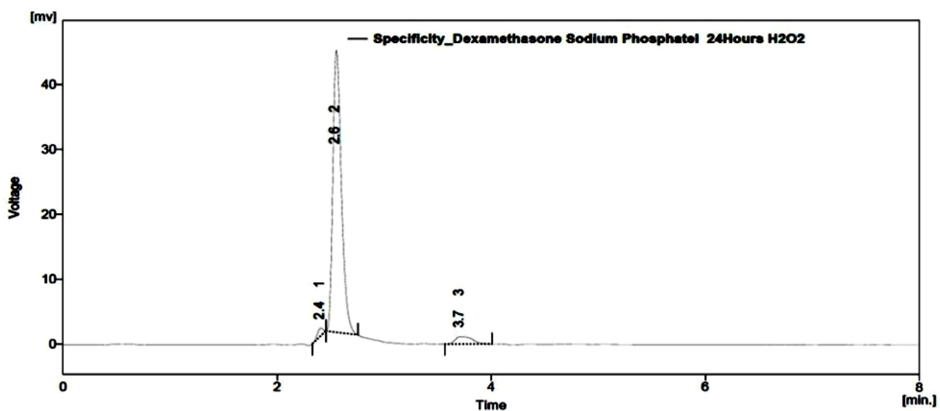

Fig. 8: Chromatogram of H_2O_2 treated DSP

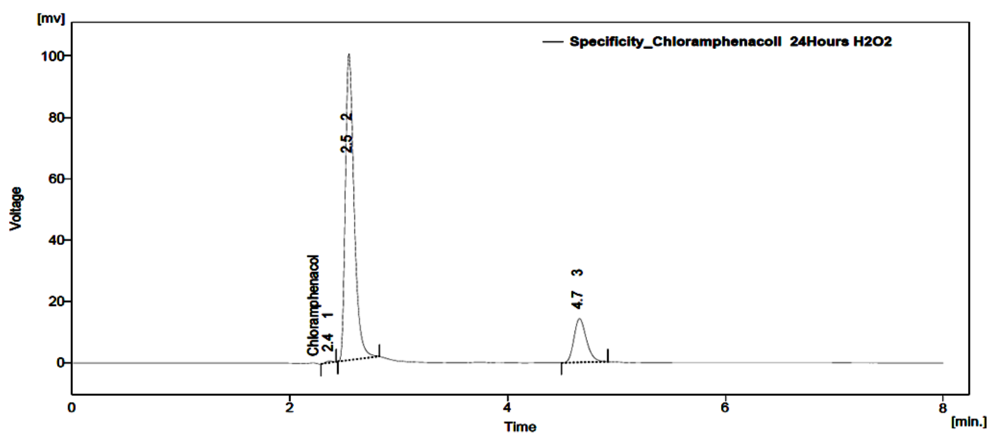

Fig. 9: Chromatogram of H_2O_2 treated CPL

Table 6: Assay of eye drops (n=6)

Drug	Label claim mg/ml	Amt. found mg/ml	Mean % recovery	% RSD*
DSP	1	1.003	100.31	0.37
CPL	5	4.98	99.63	0.47

*RSD is relative standard deviation

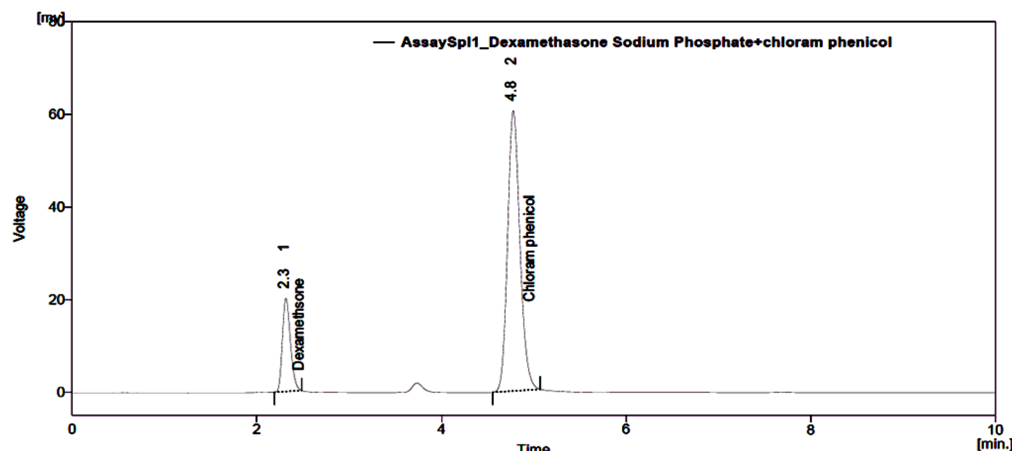

Fig. 10: Typical chromatogram for the sample solution of DSP and CPL

CONCLUSION

The method described in this paper for the simultaneous quantification of DSP and CPL was simple, specific, sensitive, accurate, precise, rapid, robust and economical. 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 are in fair agreement with the labeled amount. Thus the developed method can be proposed for routine analysis of DSP and CPL in laboratories and for quality control purposes.

REFERENCES

1. Indian Pharmacopoeia, Vol II. Published by The Indian Pharmacopoeia commission, Ghaziabad. 2007; P.1005-07.
2. Falagas ME, Grammatikos AP, Michalopoulos A. Potential old-generation antibiotics to address current need for new antibiotics. Expert Rev Anti Infect Ther. 2008; 6: 593–600.
3. Basilio M. Spectophotometric assay for Chloramphenicol and some derivatives in pure form and formulations. J Pharm Biomed Anal. 1987; 5: 577-83.
4. Satinsky D, Chocholous P, Salabova M, Solich P. Simple determination of chloramphenicol in a pharmaceutical preparation using a short monolithic column coupled to a sequential injection system. J Sep Sci. 2006; 29: 2494-99.
5. Cerkvenik- Flajs V. Performance characteristics of an analytical procedure for determining chloramphenicol residues in muscle tissue by gas chromatography-electron capture detection. Biomed Chromatogr. 2006; 20: 985-92.
6. Chen Q, Zielinski D, Chen J, Koski A, Werst D, Nowak S. A validated stability indicating HPLC method for the determination of dexamethasone related substances on dexamethasone coated drug eluting stents. J Pharm Biomed Anal. 2008; 48: 732-38.
7. Hyung WK, Donald JD. Determination of dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography. Korean J opthalmol. 1995; 9: 79-83.
8. Olga HH, Manuel JL, Elisa AC, Manuel SAL, Thea BAR. Determination of dexamethasone in urine by gas chromatography with negative chemical ionization mass spectrometry. J Chromatogr B. 2003; 788: 137-146.
9. Rele RV, Warkar CB. Simultaneous determination of ciprofloxacin hydrochloride and dexamethasone in ophthalmic solution by reversed phase high performance liquid chromatography. Asian J Research Chem. 2010; 3: 673.
10. Xiao BC, Yin LW, Ting Y. Simultaneous determination of clenbuterol, chloramphenicol and diethylstilbestrol in bovine milk by isotope dilution ultra-performance liquid chromatography–tandem mass spectrometry. J Chromatogr B. 2011; 879: 799-803.
11. Sireesha KR, Prakash K. HPLC-UV method for simultaneous determination of ofloxacin and dexamethasone sodium phosphate. Int J Pharm Pharm Sci. 2012, 4, 415-18.
12. Ashok R, Prakash PP, Tamil Selvan R. Development and validation of analytical method for estimation of prednisolone in bulk and tablets using UV- Visible spectroscopy. Int J Pharm Pharm Sci. 2011; 3 suppl 4: 184-86.
13. Iqbal, MS, Shad MA, Ashraf MW, Bilal M, Saeed M. Development and validation of an HPLC method for the determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol in presence of each other in pharmaceutical preparations. Chromatographia. 2006; 64: 219-22.
14. Shadoul WA, Gad Kariem EA, Adam ME, Ibrahim KEE. Simultaneous determination of dexamethasone sodium phosphate and chloramphenicol in ophthalmic solutions. Int. J Chem Sci Tech. 2011; 1: 60-69.
15. United States Pharmacopeia 23rd ed. The United States pharmacopeia conventions, Twin brook parkway, Rockville, MD. 1995; P.1982-84.
16. Validation of analytical procedures Q2B, 2003. International conference on harmonization, IFPMA, Geneva.