African Journal of Pharmacy and Pharmacology Vol. 5(17), pp. 1990-1995, 8 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.172
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Simultaneous determination of gatifloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations by HPLC

K. R. Sireesha[1]* and K. Prakash[2]

[1]S. N. Vanitha Pharmacy Mahavidyalaya, Exhibition grounds, Hyderabad (AP), India.
[2]Nirmala College of pharmacy, Mangalagiri, Guntur (AP), India.

Accepted 20 September, 2011

A liquid chromatography method was developed and validated for the simultaneous determination of gatifloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 5 min using a C_{18} column (250 × 4.6 mm i.d, 5 µ particle size) by isocratic elution. UV detection was carried out at 254 nm. Developed method was economical in terms of the time taken and amount of solvent consumed for each analysis. It was also validated with respect to linearity, limit of detection, limit of quantification, precision, accuracy, specificity, robustness and system suitability. The limits of detection for gatifloxacin and dexamethasone sodium phosphate were 0.397 and 0.11 µg/ml, respectively. Limits of quantification were found to be 1.203 and 0.342 µg/ml for gatifloxacin and dexamethasone sodium phosphate, respectively. The developed method was successfully applied to the simultaneous determination of gatifloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations.

Key words: Gatifloxacin, dexamethasone sodium phosphate, high performance liquid chromatography (HPLC), isocratic elution.

INTRODUCTION

Dexamethasone sodium phosphate (DSP) is a highly selective glucocorticoid which is widely used in ocular inflammatory diseases. It's chemical name is 9- fluoro-11b, 17, 21-trihydroxy-16α- methylpregna-1,4- diene-3,20-dione 21-(dihydrogen phosphate) disodium salt (Indian Pharmacoepia, 2007; Balaji et al., 2008). Gatifloxacin (GFN) is a fourth generation fluoroquinolone antibiotic used in bacterial infections. It is chemically 1-cyclopropyl-6-fluoro- 1,4- dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)- 4-oxo-3-quinoline carboxylic acid (USP, 1995; Sayed et al., 2011). Dexamethasone in combination with Gatifloxacin 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 *Staphyloccocus aureus*, *Staphyloccocus* epidermidis, *Streptococcus pneumonia* and *Haemophilus influenza.*

In the literature, methods were described for the individual estimation of fluoroquinolones and dexamethasone in aqueous samples and biological fluids by liquid chromatography (Chen et al., 2006; Hyung and Donald, 1995) liquid chromatography-fluorescence detection (Joana et al., 2011). A few methods were also given for the simultaneous determination of Dexamethasone with other drugs such as Chloremphenicol (Iqbal et al., 2006), ciprofloxacin (Rele et al., 2010) and ofloxacin (Ali et al., 2002).But simultaneous determination of these drugs in pharmaceutical formulations 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, precise and accurate HPLC method for simultaneous determination of GFN and DSP in bulk and pharmaceutical formulations. The developed method has been validated (USP, 1995; ICH Q2B 2003) by evaluation

*Corresponding author. E-mail: rupak1@rediffmail.com. Tel: 08686654459.

of the system suitability, specificity, linearity, limit of detection and quantitation, precision, accuracy and recovery. The validated method was applied to the commercially available pharmaceutical formulations containing both the drugs.

MATERIALS AND METHODS

DSP and GFN were obtained as gift samples from Ajanta Pharma Ltd, Mumbai. HPLC grade acetonitrile was purchased from SD Fine-chemicals, India. Triple distilled water was used during the study. The pharmaceutical formulations containing 3 mg/ml of GFN and 1 mg/ml DSP (ZIGAT-D eye drops, FDC proxima, India.) were 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 C18, (250 × 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 60:40 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 1 ml/min. UV detection was carried out at 254 nm.

Preparation of standard solution

Stock standard solutions of GFN and DSP were prepared in the mobile phase at a concentration of 1200 and 400 µg/ml. The working standard solutions were prepared by serial dilution of stock solutions with the mobile phase.

Sample preparation

Sample solutions of GFN and DSP were prepared at a concentration of 1200 and 400 µg/ml by diluting 10 ml of the ophthalmic solution to 25 ml with the mobile phase. From this, 1 ml was taken and diluted to 10 ml to get a concentration of 120 and 40 µg/ml of GFN and DSP.

Validation

The developed analytical method was validated as per International Conference on Harmonization (ICH) and United States Pharmacopeia (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 24 to 144 µg/ml for GFN and 8 to 48 µg/ml for DSP was prepared in triplicate and injected. Calibration curves were constructed by plotting concentration versus mean peak area.

Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) were calculated based on the standard deviation of the response and slope of the calibration curve. LOD and LOQ are calculated from the formulae 3.3 and 10 σ/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

Method precision

The precision of the method was evaluated in terms of intermediate precision, that is, intra-day and inter-day precision and by different analysts. For intra-day precision, three different concentrations of GFN and DSP in the linearity range were prepared in triplicate and were analyzed during the same day. For inter-day precision the same concentrations were analyzed on three consecutive days. The relative standard deviations (RSD) values for GFN and DSP showed that the precision of the method was satisfactory.

System precision

System precision was analyzed by injection repeatability. This was examined by analyzing six injections of the mixture containing GFN and DSP at 120 and 40 µg/ml, respectively. The RSD were calculated from the peak areas and retention times of GFN and DSP.

Accuracy

Accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amounts of GFN and DSP to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution containing GFN and DSP (1.2 and 0.4 mg/ml, respectively) was prepared by diluting 10 of the ophthalmic solution to 25 ml in volumetric flask and make-up of the solution with the mobile phase, samples (0.4 ml) of the filtered solution were transferred to 10 ml volumetric flasks containing 0.4, 0.6 and 0.8 ml of GFN and DSP standard solution, dilutions were made and analyzed. The percentage recovery and the percentage RSD was calculated and found to be within the limits.

Specificity

Specificity of an analytical method may 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.

Robustness

Robustness of the method is a measure of capacity of the method to remain unaffected by small but deliberate variations in method parameters and provides an indication of the variability during normal usage. Robustness of the method was evaluated by 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 1 ml/min to 1 ± 0.1 ml/min. Effect of these changed parameters was studied by injecting the sample into the system.

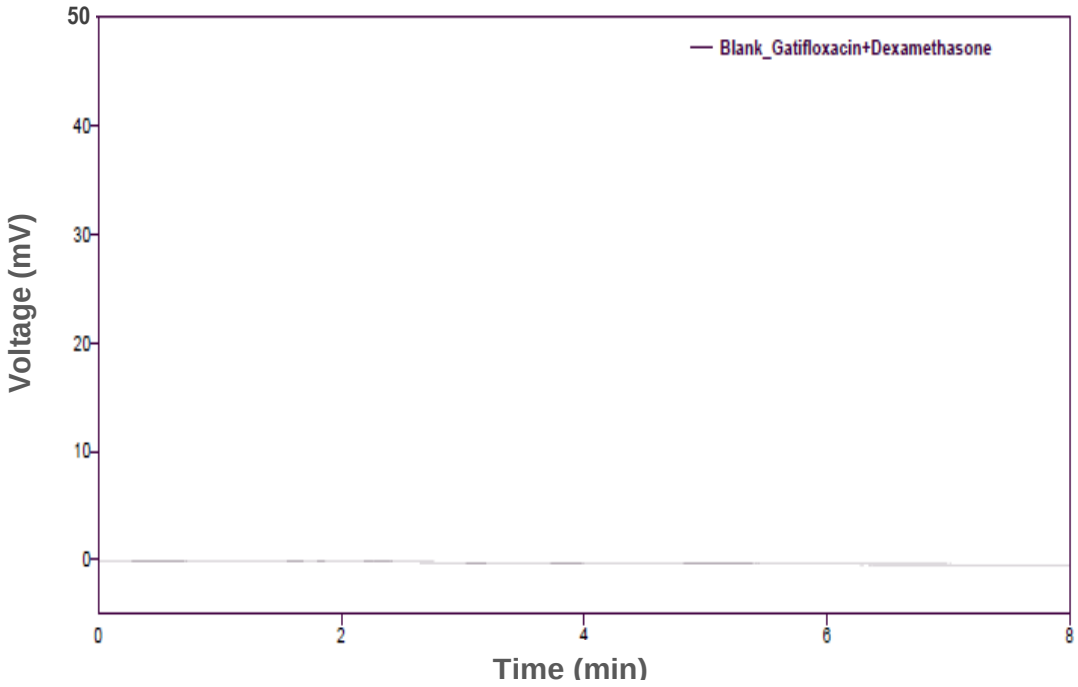

Figure 1. Chromatogram for blank.

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 and plate number were investigated.

Assay of the marketed formulation

The developed method was applied to the simultaneous determination of GFN and DSP 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 GFN and DSP with short analysis time (< 5 min) and acceptable resolution (RS > 2). 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 the ratio of 60:40 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 (Figure 1) and shows no interference with the drug peaks. The optimum wavelength for detection was set at 254 nm at which better detector response for both drugs was obtained. The retention times were 2.42 and 4.81 min for GFN and DSP, respectively (Figure 2).

Validation

Calibration graphs were constructed by plotting the peak area versus their corresponding concentrations. Good linearity was obtained in the range of 24 to 144 µg/ml and 8 to 48 µg/ml for GFN and DSP. The results are shown in Table 1. LOD and LOQ were calculated from the slope and standard deviation y-intercepts of the regression line of the calibration curve. For GFN it was found to be 0.397 and 1.203 µg/ml and for DSP 0.11 and 0.342 µg/ml, respectively. The precision of the method and instrument precision was evaluated and relative standard deviation (RSD) values were calculated. The RSD values for GFN and DSP 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 GFN and DSP; the results are as shown in Table 3. Developed method was found to be robust when the detection wavelength and flow rate was changed from 254 to 254 ± 2 nm and 1 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 GFN and DSP were found to be 2.69 and 5.34 min, respectively and with 1.1 ml/min flow rate, retention times for GFN and DSP were found to be 2.21 and 4.41 min, respectively

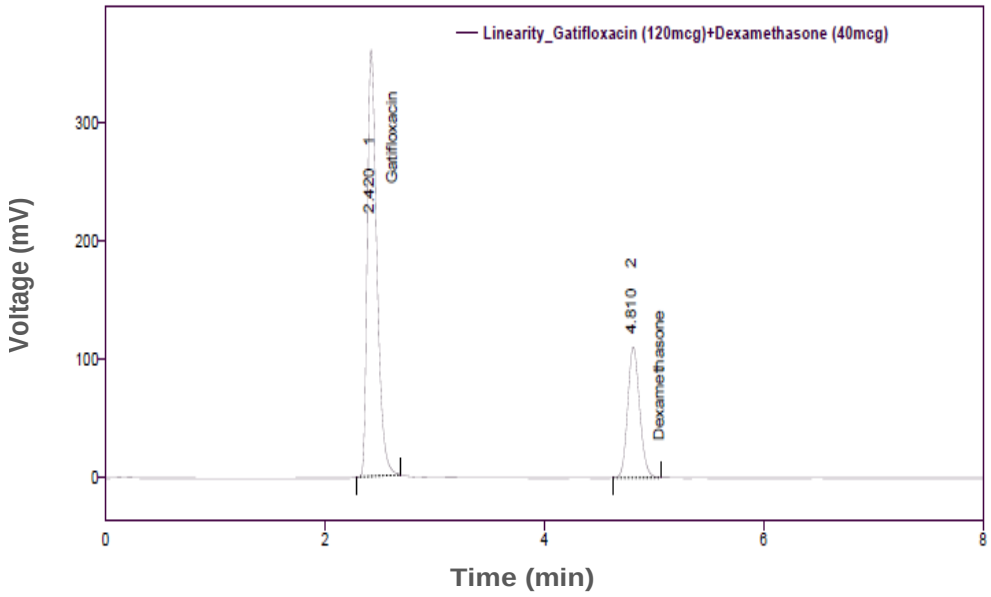

Figure 2. Typical chromatogram for the standard solution of GFN and DSP.

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

Substance	R^2	Slope	Concentration range (µg/ml)
GFN	0.999	17.94	24-144
DSP	0.999	20.56	8-48

'n' is the number of determinations.

Table 2. Precision (% RSD).

Parameter	GFN	DSP
Intra-day precision	0.033	0.096
Inter-day precision	1.78	1.39
Analyst precision	0.08	0.19
Injection repeatability for t_R	0	0.027
Injection repeatability for peak area	0.12	0.22

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

Table 3. Recovery studies (n = 6).

Drug	Concentration (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	RSD (%)
GFN	96	95.94	99.94	0.037
	120	119.65	99.71	0.04
	144	143.86	99.9	0.052
DSP	32	31.97	99.26	0.05
	40	39.86	99.65	0.426
	48	45.85	99.68	0.29

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

Table 4. System suitability parameters (n = 6).

Parameter	GFN	DSP
Retention time (t_R)	2.42	4.81
Asymmetry factor	1.7	1.2
Resolution	-	13.2
Number of plates	3735	8926

'n' is the number of determinations.

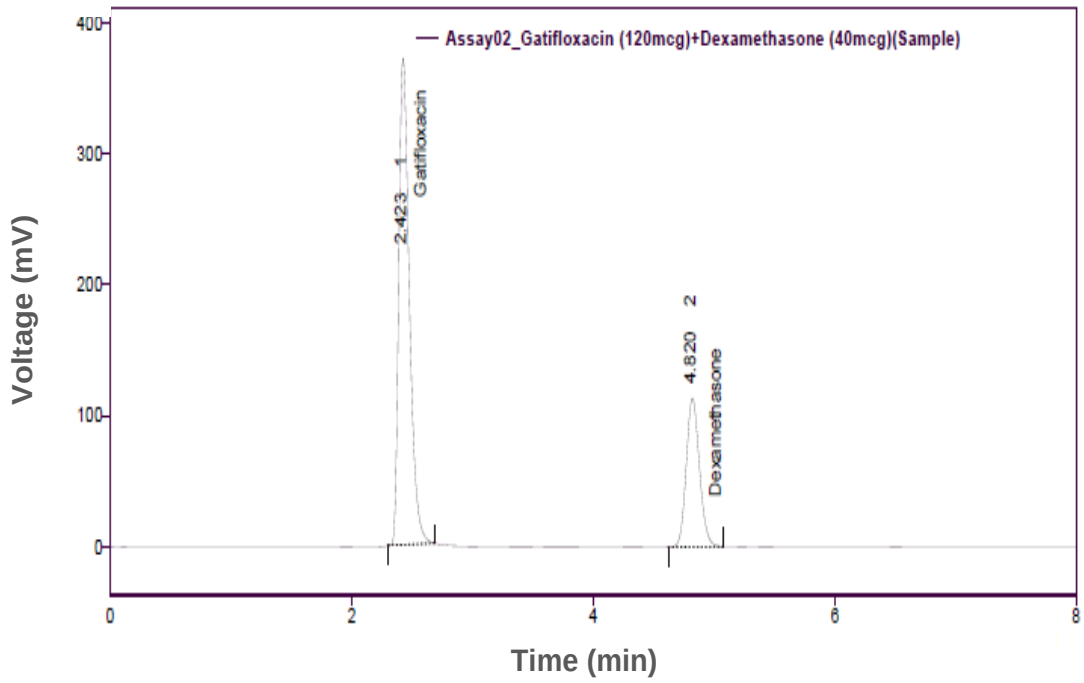

Figure 3. Chromatogram for the sample solution of GFN and DSP.

Table 5. Assay of eye drops (n = 6).

Drug	Label claim (mg/ml)	Amount found (mg/ml)	Mean recovery (%)	RSD (%)
GFN	3	3	99.7 ± 0.8	0.034
DSP	1	0.99	99.44 ± 0.4	0.032

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

without affecting the resolution of the drugs. When detection wavelength was changed to 254 ± 2 nm, the retention time for GFN and DSP were not changed from the normal. System suitability parameters are shown in Table 4.

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 found to be within the limits. The low RSD value indicated suitability of this method for routine analysis of GFN and DSP in pharmaceutical dosage forms. Chromatogram of the sample shows that there was no interference from the excipients present in the formulation (Figure 3); 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 GFN and DSP are found 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 GFN and DSP in laboratories and for quality control purposes.

REFERENCES

Ali M.S, Ghori M, Saeed A (2002). Simultaneous Determination of Ofloxacin, Tetrahydrozoline Hydrochloride, and Prednisolone Acetate by High-Performance Liquid Chromatography., J. Chromatographic Sci., 40(8): 429-433.

Balaji K, Raghunatha reddy GV, Madhusudan reddy K, Jayaramireddy S (2008). Determination of prednisolone, dexamethasone and hydrocortisone in pharmaceutical formulations and biological fluid samples by voltammetric techniques using β- cyclodextrin modified carbon paste electrode. Afr. J. Pharm. Pharmcol., 2(8): 157-166.

Chen Y, Zhou YP, Tan DH, Deng JJ, Xu LF (2006). Simultaneous Determination of Ciprofloxacin hydrochloride and Dexamethasone Acetate in Compound Ciprofloxacin Hydrochloride Ear Drops by HPLC. J. Liaoning Univ., 4: 10.

Hyung WK, Donald JD (1995). Determination of Dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography. Korean J. Opthalmol., 9: 79-83.

Indian Pharmacopoeia, Vol II (2007). Published by The Indian Pharmacopoeia commission, Ghaziabad, 1158: 1005-1007.

Iqbal MS, Shad MA, Ashraf MW, Bilal M, Saeed M (2006). 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, 64: 219-222.

Joana S, Gilberto A, Ana F, Angelina P, Celeste L, Amilcar F (2011). Development and validation of a fast isocratic liquid chromatography method for the simultaneous determination of norfloxacin, lomefloxacin and ciprofloxacin in human plasma. Biomed. chromatogr., 25: 535-41.

Rele RV, Warkar CB (2010). Simultaneous Determination of Ciprofloxacin Hydrochloride and Dexamethasone in Ophthalmic Solution by Reversed Phase High Performance Liquid Chromatography. Asian J. Res Chem., 3(3): 673

Syed MR, Nadia MSA, Mansour ME (2011). Evaluation of effects of ciprofloxacin and gatifloxacin on neurotransmitter levels on cortex and hippocampus. Afr. J. Pharm. Pharmacol., 5(8): 993-1005.

United States Pharmacoepia 23 (1995). The United States pharmacoepial convention, twinbrook parkway, Rockville, MD. 472-473.