

HPLC-UV method for simultaneous determination of sparfloxacin and dexamethasone sodium phosphate in eye drops

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Abstract: A simple, sensitive liquid chromatographic method was developed and validated for the simultaneous estimation of sparfloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 10 min using a C₁₈ 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 (50:50, v/v) was used. Column effluents were monitored at 224nm at a flow rate of 1ml/min. Retention times of sparfloxacin and dexamethasone sodium phosphate were 3.01 and 6.47 min respectively. The linearity of sparfloxacin and dexamethasone sodium phosphate was in the range of 3-18μg/ml and 1-6μg/ml respectively. Developed method was economical because, the time taken and amount of solvent consumed for each analysis was less. The method was validated and was applied to the simultaneous determination of sparfloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations.

Keywords: Simultaneous determination, HPLC, isocratic elution, validation.

INTRODUCTION

Sparfloxacin (SFN) is a third generation fluoroquinolone antibiotic used in bacterial infections. It is chemically (cis) - 5-amino-1-cyclopropyl-7-[(3, 5-dimethyl piperazin-1-yl) -6, 8-difluoro- 1, 4-dihydro 4-oxo-quinoline- 3-carboxylic acid (Merk index, 2001). Dexamethasone sodium phosphate (DSP) 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 (The Indian Pharmacopeia commission, 2007). Dexamethasone in combination with sparfloxacin is used in several anti-infective eye preparations to treat acute and sub acute conjunctivitis, keratitis and corneal ulcers caused by susceptible strains of the following aerobic gram positive and negative bacteria such as *S. aureus*, *S. epidermidis*, *S. pneumonia* and *Haemophilus influenza* (Vyas *et al.*, 2002).

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.*, 2008; Hyung *et al.*, 1995) liquid chromatography-fluorescence detection (Joana *et al.*, 2011). A few methods were also given for the simultaneous determination of Dexamethasone and sparfloxacin with other drugs such as Chloremphenicol (Iqbal *et al.*, 2006), ciprofloxacin (Rele and Warkar, 2010) ofloxacin (Tang *et al.*, 2002) and some H₂ receptor antagonists (Najma *et al.*, 2011). But simultaneous determination of SFN and DSP 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 determination of SFN and DSP in bulk and pharmaceutical formulations simultaneously. The developed method has been validated (The United States Pharmacopeia Convention, 1995; Validation of Analytical Procedures Q2 B, 2003) 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

DSP and SFN 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 3mg/ml of SFN and 1mg/ml DSP 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 Chromosil C₁₈ column (250 mmx4.6 mm i.d, 5μ particle size) was used.

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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 50:50, 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 μ l and the flow rate was 1 ml/min. UV detection was carried out at 224 nm. Chromatographic separations were carried out at room temperature (25-30°C).

Preparation of solutions

Preparation of standard solution

Stock standard solutions of SFN and DSP were prepared by dissolving respective standards in the mobile phase to get a concentration of 600 μ g/ml and 200 μ g/ml. Working standard solutions were prepared by suitable dilutions of stock solution with the mobile phase.

Preparation of sample solution

Sample solutions of SFN and DSP were prepared at a concentration of 600 μ g/ml and 200 μ g/ml by diluting 5 ml of the ophthalmic solution to 25 ml with the mobile phase. From this 0.25 ml was taken and diluted to 10 ml to get a concentration of 15 μ g/ml and 5 μ g/ml of SFN and DSP respectively.

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 3-18 μ g/ml (3, 6, 9, 12, 15, 18 μ g/ml) for SFN and 1-6 μ g/ml (1, 2, 3, 4, 5, 6 μ g/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 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 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 SFN 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 different days over a period of one week and RSD values were calculated. Instrument precision was analyzed by injection repeatability. This was examined by analyzing six injections of the mixture containing 15 and 5 μ g/ml of SFN and DSP, respectively. RSD values were calculated from the peak areas and retention times of SFN and DSP.

Accuracy

Accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amounts of SFN and DSP to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution containing SFN and DSP (0.6 and 0.2 mg/ml, respectively) was prepared by diluting 5 ml of the ophthalmic solution to 25 ml in a volumetric flask, and make up the solution with the mobile phase. Samples (0.1 ml) of the filtered solution were transferred to 10 ml volumetric flasks containing 0.1, 0.15, and 0.2 ml of SFN and DSP 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 concentration of analyte in presence of all other sample materials such as matrix, degradation products and other related substances. Sample solution 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 224 nm to 224 $\pm$ 2 nm and flow rate was changed from 1 ml/min to 1 $\pm$ 0.1 ml/min. Effect of these changed parameters was studied by injecting the sample into the system.

System suitability

System suitability was established in order to ensure that the developed method can generate results of acceptable accuracy and precision. Parameters including retention factor, asymmetry factor / tailing factor, resolution and plate number were used to determine system suitability.

Assay of the marketed formulation

The developed method was applied to the simultaneous determination of SFN and DSP in pharmaceutical formulations. Sample was analyzed by performing six independent determinations and each series was injected in triplicate.

RESULTS

Mobile phase optimization

Chromatographic conditions were optimized to develop a HPLC method for simultaneous estimation of SFN and

DSP with short analysis time (<10min), and acceptable resolution ($R_s > 2$). 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 50:50 at a flow rate of 1ml/min, symmetrical peaks with good resolution were obtained. The optimum wavelength for detection was set at 224nm at which a good detector response was obtained for both drugs. The retention times were 3.01 and 6.47min for SFN and DSP respectively (fig. 2).

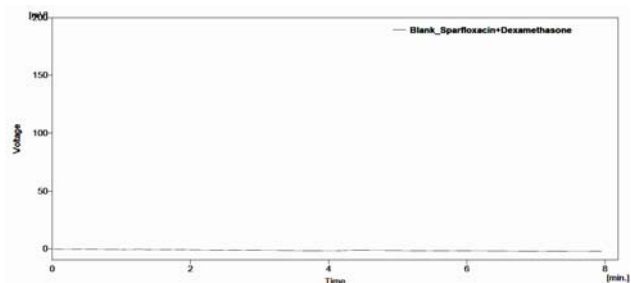


Fig. 1: Chromatogram for blank

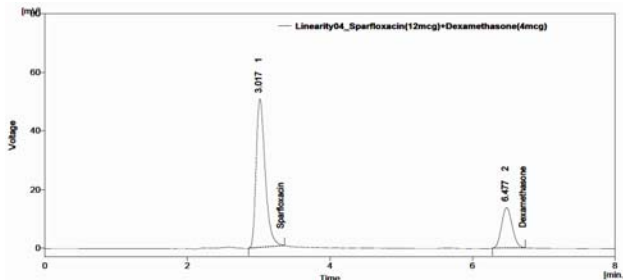


Fig. 2: Typical chromatogram for the standard solution of SFN and DSP

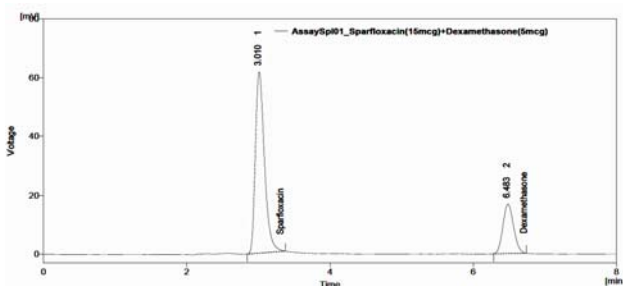


Fig. 3: Chromatogram for the sample solution of SFN and DSP

Validation

Calibration graphs were constructed between the peak areas versus their corresponding concentrations. Good linearity was obtained in the range of 3-18 $\mu\text{g/ml}$ and 1-6 $\mu\text{g/ml}$ for SFN and DSP. 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 SFN it was found to be 0.0353 and 0.1071 $\mu\text{g/ml}$ and for DSP 0.023 and 0.072 $\mu\text{g/ml}$ respectively. The precision of the method and instrument precision was evaluated and expressed as relative standard deviation (RSD). Precision of the method was

determined by calculating RSD's from the peak area of the standard drug solutions in the concentration range. Instrument precision was determined by two parameters like injection repeatability for retention time and injection repeatability for peak area. The results are shown in table 2. The accuracy of the method was established by recovery studies. The amount extracted at each concentration was calculated and compared with the total amount of the sample and standard drug added before extraction. Good recoveries were obtained and were found to be between 99-101% for both SFN and DSP; the results are given in the table 3. Developed method was found to be robust when the detection wavelength and flow rate was changed from 224 nm to 224 ± 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 SFN and DSP were found to be 3.31 and 6.89 min respectively and with 1.1 ml/min flow rate, retention times for SFN and DSP were found to be 2.84 and 6.02 min, respectively without affecting the resolution of the drug components. When detection wavelength was changed to 224 ± 2 nm, the retention time for SFN and DSP were not changed from the normal. System suitability parameters are shown in table 4. The assay value of the marketed formulation was determined and RSD values were calculated. Sample recoveries were found to be good and it was 100.4% for SFN and 99.9% for DSP. The results are given in table 5.

DISCUSSION

The regression equations were calculated as $Y = 37.12X + 0.143$ for SFN and $Y = 36.05X - 0.927$ for DSP. The correlation coefficient (R^2) was greater than 0.999 in both the cases. Hence the method is linear in the stated range. Precision determines degree of repeatability of an analytical method. More over the repeatability of a method can be assessed at different levels and through different parameters to ensure its validity. The RSD values for SFN and DSP showed that the precision of the method was satisfactory. By deliberate variations in method parameters such as detection wavelength and flow rate robustness of the method was established. To verify the developed method, assay of the marketed formulation was carried out. 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 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. fig. 3 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. System suitability parameters are vital part of analytical procedures and these were established by different parameters which proves the integrity of the developed analytical methodology.

Table 1: Linearity by regression analysis

Conc. SFN ($\mu\text{g/ml}$)	Mean area* $\pm$ S.D	%RSD	Conc. DSP ($\mu\text{g/ml}$)	Mean area* $\pm$ S.D	%RSD
3	114 $\pm$ 2.26	1.9	1	36.1 $\pm$ 0.78	2.17
6	217.3 $\pm$ 3.05	1.4	2	66.5 $\pm$ 1.25	1.88
9	341.34 $\pm$ 0.75	0.22	3	106.9 $\pm$ 0.92	0.86
12	445.5 $\pm$ 2.08	0.47	4	142.59 $\pm$ 1.14	0.8
15	554.23 $\pm$ 2.81	0.5	5	176.14 $\pm$ 2.25	1.28
18	664.9 $\pm$ 4.5	0.68	6	214.8 $\pm$ 3.6	1.66

*Mean of six determinations

Table 2: Precision expressed as %RSD

Parameters	SFN*	DSP*
Intra-day precision	0.253	0.017
Inter-day precision	0.467	1.46
Analyst precision	0.12	0.18
Injection repeatability for t_R	0.34	0.09
Injection repeatability for peak area	0.64	0.18

Table 3: Recovery studies (n=6)

Drug	Concentration $\mu\text{g/ml}$	Amount recovered $\mu\text{g/ml}$	% recovery*	% RSD*
SFN	12	12.01	100.05	0.824
	15	15	100	0.182
	18	17.92	99.57	0.305
DSP	4	4.03	100.82	0.505
	5	4.98	99.67	0.39
	6	6.04	100.68	0.49

Table 4: System suitability parameters (n=6)

Parameters	SFN	DSP
Retention time (t_R)	3.01	6.47
Asymmetry factor	1.63	1.19
Resolution	-	13.57
Number of plates	2723	8711

'n' is number of determinations

Table 5: Assay of eye drops

Drug	Label claim mg/ml	Amt. found mg/ml	Mean* % recovery	% RSD*
SFN	3	3.01	100.4	0.13
DSP	1	0.99	99.9	0.268

*Mean of six determinations and RSD is relative standard deviation

CONCLUSION

The method described in this paper for the simultaneous estimation of SFN and DSP was found to be simple, 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 SFN and DSP in laboratories and for quality control purposes.

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