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DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR ESTIMATION OF SPARFLOXACIN IN TABLET DOSAGE FORM

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ABSTRACT: The study describes development and subsequent validation of a reverse phase (RP) high-performance liquid chromatography (HPLC) method for the estimation of sparfloxacin (SPR) in tablet dosage form. The liquid chromatographic separation was achieved isocratically using a mobile phase composed of phosphate buffer (5.3 mM) and acetonitrile in the ratio of 60:40 (v/v) adjusted to pH 3.5 using dilute phosphoric acid. The analysis was carried out using X terra column (4.6 × 150 mm, 5µm, C_{18}) at a flow rate of 0.5 ml/min and the UV detection at 298 nm. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness and selectivity. The linearity of the proposed method was investigated in the range of 4 to 24 µg/ml (r^2 =0.999). Quantitative and recovery studies of the dosage form were also carried out and analyzed, the % RSD from recovery studies was found to be within the limit. Due to simplicity, rapidity and accuracy of the method we believe that the method will be useful for routine quality control analysis.

INTRODUCTION: Sparfloxacin is a long acting broad spectrum synthetic antibacterial agent belonging to the class Fluoroquinolone. It is used for the treatment bacterial infections like other fluoroquinolones. It is a DNA-gyrase and topoisomerase IV inhibitor used for the treatment of urinary tract and respiratory tract infections. It is also used for the treatment of bacterial infection in eyes [1]. The drug is found to be effective in community acquired pneumonia [2], drug resistant tuberculosis [3], skin and soft tissue infection [4] and complicated urinary tract infections [5].

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The drug is found to be effective against a wide range of gram positive and gram negative bacterial, glucose non-fermentors and anaerobes, *Legionella*, *Mycoplasma*. *Chlamydia*, *Mycobacterium* species, Methicillin-resistant *Staphylococcus aureus* [6].

Chemically sparfloxacin is 5-amino-1-cyclopropyl-7-[(3*R*,5*S*)3,5-dimethylpiperazin-1-yl]-6,8-difluoro -4-oxo-quinoline-3-carboxylic acid as presented in **Figure 1**.

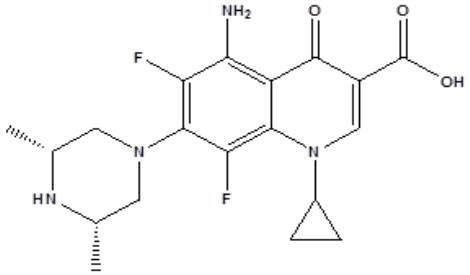

FIGURE 1: CHEMICAL STRUCTURE OF SPARFLOXACIN

The molecule has got poor aqueous solubility resulting poor G.I. tract absorption. Its biological half-life is 15 to 22 hours in healthy volunteers after oral administration [7]. The molecule gets well distributed throughout the body with a higher concentration in sinus mucosa, bronchial mucosa, epithelial lining fluid, and alveolar macrophages [8] and is the drug of choice for treating the lower respiratory tract infection.

Extensive literature survey presented a fewer number of suitable analytical method for the estimation of Sparfloxacin from oral dosage forms and ophthalmic preparations. Only few spectro-photometric methods [9, 10] and chromatographic [11-13] methods have been reported. A rapid, precise, sensitive chromatographic method for the estimation of Sparfoloxacin from oral dosage forms is therefore essential. Reported methods are somewhat tedious and time consuming [9-13].

In our current study, a validated rapid analytical method for the estimation of Sparfloxacin has been developed using reverse phase chromatographic technique.

EXPERIMENTAL:

Reagents and chemical: Potassium di-hydrogen phosphate and dipotassium hydrogen phosphate of AR grade, phosphoric acid and acetonitrile of HPLC grade were purchased from Merck Ltd., Mumbai. Reference standard of sparfloxacin was procured from Central Drug laboratory, Kolkata. Tablet of brand Novospar tablet (Zydus Cadila) having of label claim of sparfloxacin 200mg was used. Water of HPLC grade was obtained from Aurium 611 UV water purifier of Sartorius, Germany.

Equipment: Chromatographic separation was performed on Waters Alliance e2695 HPLC module having 2489 dual Lambda UV absorbance detector. Empower 3 software was applied for data collecting and processing.

HPLC conditions: A C_{18} X terra column (150 mm × 4.6 mm, 5 µm) was used for separation. Mobile phase consisting of a mixture of 60 volumes of 5.3 mM phosphate buffer solution adjusted to pH 3.5 ± 0.1 with orthophosphoric acid and 40 volumes of accetonitrile was pumped at 0.5 ml /min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. The eluents were monitored at 298 nm. The injection volumes of sample and standard were 10 µl.

Standard preparation: Standard stock solution of 0.5 mg/ml of sparfloxacin was prepared in 10 ml HPLC grade water by addition of 1 drop of conc. HCl followed by sonication for 15 min and the final volume was made by the mobile phase. The final working standard solution of sparfloxacin had a final concentration of 0.02 mg/ml and was prepared by appropriate dilution with mobile phase from the stock solution. The content of standard solution was filtered through 0.45 µm syringe filter.

Sample preparation: Twenty tablets were weighed and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10.0 mg of sparfloxacin was transferred into a 25 ml volumetric flask. About 10 ml of water and 1 drop of conc. HCl was added to the volumetric flask and sonicated for 15 minutes and volume was made up to the mark by mobile phase. This solution was filtered through Whatman No. 1 and further diluted to 0.016 mg/ml of sparfloxacin (theoretical value) in mobile phase. The content of sample solutions was filtered through 0.45 µm syringe filter.

Method validation: The developed method was validated as per ICH guidelines in terms of parameters like specificity, precision, accuracy, linearity and range, LOD, LOQ, ruggedness, robustness etc.[14, 15].

System suitability: System suitability tests were carried out on freshly prepared solution of reference standards of sparfloxacin to check various parameters like retention time, tailing factor, theoretical plate etc. The results were shown in **Table 1**.

TABLE 1: SAMPLE SUITABILITY PARAMETER

Parameters	Sparfloxacin
Wavelength of the max absorbance (nm)	298.0
Retention Time (mins)	2.528
Tailing factor	0.5123
Theoretical Plate	12156
R.S.D of multiple injection (Amount, six replicates)	0.01
LOD (µg/ml)	0.05
LOQ (µg/ml)	0.29

Linearity and range: The linearity was studied to determine the range over which analyte response in a linear function of concentration. The study was performed by preparing standard solutions of the sparfloxacin at five different concentrations and analyzes were performed in triplicate. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Table 2**. The linearity curve of sparfloxacin was represented in **Figure 2**.

TABLE 2: LINEARITY PARAMETERS

Parameters	Sparfloxacin
Linearity range (µg/ml)	4.0 to 32
Regression coefficient	0.999
Slope	54017
Intercept	8703

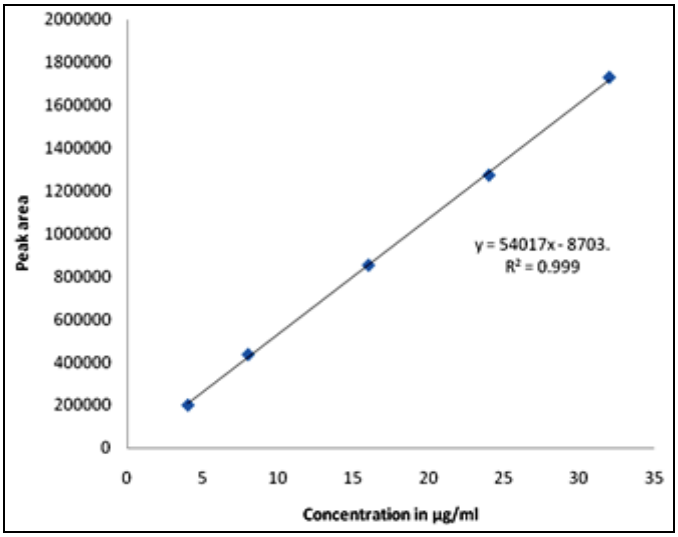

FIGURE 2: SOLUTION CONCENTRATION VERSUS PEAK AREA LINEARITY CURVE

Precession: The precession of an analytical method in the closeness of replicate results obtained from analysis of the same homogeneous sample. The intra-day precision of the proposed method was determined by making six replicate injections of the standard solution on the same day. Inter-day precision was evaluated by analysing the same sample in the same way on different days. The percentage RSD with respect to the peak area, peak retention time and amount were calculated for each case and presented in **Table 3**. The lower values of %RSD prove that the method is precise [14].

Assay: 10 µl standard and sample solutions were injected on HPLC system using optimized mobile phase and other chromatographic conditions. Chromatograms of standard solutions (six replicates) and sample solutions (two replicates) were recorded. Typical chromatogram of sparfloxacin was presented in **Figure 3**. The concentration of sparfloxacin in the tablet formulation was calculated by comparing area of the sample with that of the standard. The percentage assay of the drug was calculated and presented in **Table 4**.

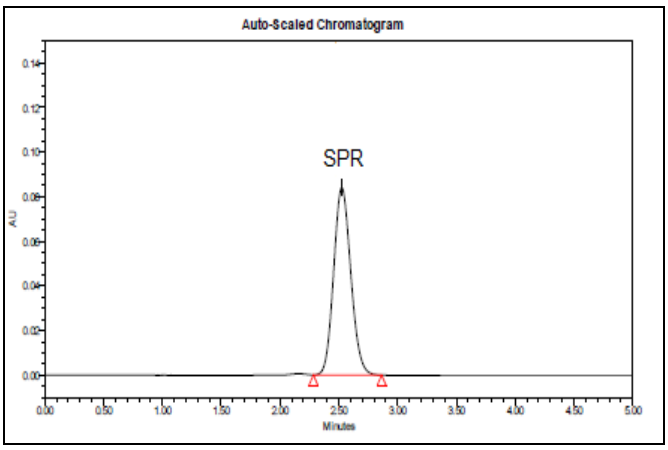

FIGURE 3: REPRESENTATIVE CHROMATOGRAM FOR SPR (RETENTION TIME = 2.528)

Accuracy: The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. To evaluate the accuracy of the proposed method, recovery tests were carried out. Recovery tests were performed by adding known amounts of standard solutions (90%, 110% and 120%) to the pre-analysed sample followed by analysis using the proposed method. The recovery studies were performed in triplicate and percentage recovery and standard deviation of the percentage recovery were calculated and presented in **Table 5**. The lower values of %RSD of assay indicate the method is accurate and there is no interference from excipients [14, 15].

TABLE 3: PRECISION PARAMETERS

Parameters	Intra-day	Inter-day			
		Day1	Day2	Day3	Mean
Peak Area	853514	853489	853498	853409	853465
Peak RT	2.528	2.526	2.524	2.525	2.525
Amount (mg/Tablet)	199.98	199.97	199.97	199.95	199.96

TABLE 4: ASSAY PARAMETERS

Tablet Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated		
Sparfloxacin	SPR	200	199.98	99.99	0.57

TABLE 5: ACCURACY PARAMETERS (RECOVERY STUDIES)

Tablet Formulation	Drug	Labelled Amount of Drug (mg/tab)	Amount mg/tab found	% label claim (n =6)	Recovery Studies (n = 3)				
					Total Amt. after spiking (mg)	Amount recovered (mg) Mean ± SD	% Recovery	% Mean Recovery	% RSD
Sparfloxacin Tab Novospar tab (Zydus Cadila)	SPR	200	199.98	99.99	180 220 240	179.23±2.11 219.01±1.99 241.00±1.59	99.57 99.55 100.42	99.85	0.49

Limit of detection and limit of quantification: The limit of detection (LOD) in the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3) and the limit of quantitation (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution using the developed RP-HPLC method. The results were shown in Table 1.

Ruggedness and robustness: The ruggedness of the proposed method was determined by carrying out the experiment on different instruments like Merk Hitachi HPLC La Chrome pump L-7100 with Merk Hitachi UV La Chrome detector L-7400, Waters HPLC 600 pumps by different operators using different columns of similar type [14].

Robustness of the method was determined by making slight changes in the Chromatographic conditions, such as change in mobile phase, flow rate and column temperature. It was observed that there were no marked changes in the chromatograms and did not affect the recovery of the drug which indicated that the proposed method is rugged and robust.

The robustness limit for mobile phase variation, flow rate variation and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

RESULTS AND DISCUSSION: In order to validate our proposed method, parameters such as detection wavelength, ideal mobile phase, optimum pH and concentrations of the standard solutions were exhaustively studied. Of several solvents and solvent mixtures investigated, the mobile phase consisting of phosphate buffer (5.3 mμ) and acetonitrile in the ratio of 60:40 (v/v) adjusted to pH 3.5 was found to furnish sharp, well defined peaks with very good symmetry (Fig. 2) at wavelength 298 nm. The proposed method is simple and do not involve laborious time consuming sample preparation.

The proposed method is a cost effective or economical method because a flow rate of 0.5 ml /min was selected after preliminary test with retention time 2.528. The method was statistically validated as per ICH guideline. The analytical procedure was found to be linear in the concentration range 4 to 32µg/ml with regression factor 0.999. The percent RSD with respect to peak area, peak retention time and amount for sample solution were 0.06, 0.09 and 0.12 respectively and the values were 853514, 2.528 and 199.98mg/Tab respectively for intraday precision and 853465, 2.525 and 199.96mg/Tab respectively for interday precision (Table 3), indicating precision of the method. The recovery values obtained were between 99.55 % and 100.42% (Table 5), confirming accuracy of the proposed method.

The LOD and LOQ were found to be 0.5317µg/ml and 1.6111µg/ml respectively. The method was satisfactory with respect to ruggedness and robustness also.

CONCLUSION: The proposed HPLC method enables quantitative determination of sparfloxacin. The proposed method was found to be easy in preparation of sample, fast, precise, accurate, sensitive, efficient and economical and can be used in routine analysis in quality control laboratories.

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