Research Article

DEVELOPMENT AND VALIDATION OF A GREEN BIOANALYTICAL METHOD FOR THE DETERMINATION OF SPARFLOXACIN IN PHARMACEUTICAL DOSAGE FORM AND HUMAN PLASMA BY RP-HPLC

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ABSTRACT

This study describes the development of an innovative, green, rapid, precise, selective and sensitive reverse phase high-performance liquid chromatography method for the quantitative determination of Sparfloxacin (SPR) in human plasma and pharmaceutical dosage form**.** Extraction of drug from plasma was done by employing optimized liquid-liquid extraction procedure**.** The sample was analyzed using Methanol: Water (pH-7 with triethylamine) (60:40 % v/v) as mobile phase. Chromatographic separation was achieved on Prontosil C-18 column (4.6 x 250mm, 5µ particle size) as stationary phase using isocratic elution (at a flow rate of 1 ml/min). The peak was detected using UV-PDA detector set at 254 nm and the total time for a chromatographic separation was 9 min. The calibration curve obtained was linear (r^2= 0.9998) over the concentration range of 5-25µg/ml. Method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.573 and 1.542 µg/ml respectively. There was no significant difference between the amount of drug spiked in plasma and the amount recovered and plasma did not interfere in estimation**.** Other important factors is that the method uses less amounts of organic solvent, produces low levels of waste and does not use buffer solution, minimizing effluent treatment, which contributes to the environment and implements methods aimed green chemistry, making economic for the industry. Thus, the proposed method is suitable for the analysis of SPR in tablet dosage forms and human plasma**.**

Keywords: RP-HPLC, Green, Sparfloxacin, Human plasma, Liquid-liquid extraction

INTRODUCTION

Synthetic sparfloxacin (SPR, cis-5-amino-1-cyclopropyl-7-(3,5-dimethylpiperazin-1-yl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, Figure 1), is the third generation broad spectrum floroquinolone antimicrobial agent for oral administration and has good to excellent efficacy against Gram positive cocci (notably *S. pneumoniae*) and shows selective action against anaerobes and atypical pathogens. It is also moderately active against some (*B. fragilis* group) L. mono-cytogenes resistant[1-6]. It is official in martindale extra pharmacopeia[7]. Sparfloxacin, a potent inhibitor of bacterial DNA gyrase, is bactericidal at or near the minimum inhibitory concentration[8] and commonly prescribed for infective opthalmitis and sinusitis, acute exacerbation of chronic bronchitis, community-acquired pneumonia, eye infections, urinary tract infection[9]. Several analytical methods that have been reported for the estimation of sparfloxacin alone or combination with other drugs in pharmaceutical formulations and/or biological fluids include spectrophotometric[10-12], spectrofluorometry[13], high-performance liquid chromatography (HPLC)[14-21], stability indicating[22], HPTLC[23,24], colorimetric[25], LC-MS/MS[26,27], UPLC[28] and immunoassay[29]. Although several methods have been reported previously in the literature for determination of sparfloxacin in variety of sample, among them many articles have described the use of buffers to develop RP-HPLC method. The use of buffer as a mobile phase in HPLC has

many limitations as it requires filtration before use, crystallization may occur when the organics come in contact with left behind buffers in system which may damage the pump and column and also produce corrosion of stainless steel lines. Besides these buffers require to prepare daily because especially phosphate buffer is a good medium for bacterial and fungal growth. To overcome the limitations of buffers, the objective of this work was to develop green, accurate and rapid liquid chromatographic analytical method for the determination of sparfloxacin in pharmaceutical formulations and plasma. The method doesn't require various elaborate treatments and tedious extraction procedures. The developed method can be applied successfully to quality control and for other analytical purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines[30], which is mandatory also.

MATERIALS AND METHODS

The pure drug sample of SPR (99.5%) was obtained as gift sample from FDC Limited, Mumbai, India. Milli -Q-water was used throughout the process, HPLC grade acetonitrile, methanol was purchased from Merck Ltd (Mumbai, India). All other chemicals and reagents used were of analytical grade only. The human plasma was received from Peoples Hospital, Bhopal, M.P., India. Novospar (200mg) Tablet was purchased from the local market.

Equipments

A high performance liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector (YL 9160 detector) connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used.

Chromatographic Conditions

The chromatographic analysis was performed by using a mobile phase of Methanol: Water (pH-7 with triethylamine) (60:40 % v/v). These were filtered through 0.45μ membrane filter and degassed by sonication before use. The mobile phase was pumped isocratically at a flow rate of 1.0ml/min during analysis at ambient temperature. The retention time for SPR was observed to be 7.924 ± 0.3 min. Total time of analysis was less than 9 min and the volume of injection was $20\mu l$ and eluent was detected at 254 nm on a Prontosil C-18 column (4.6 x 250mm, 5μ particle size).

Selection of Mobile Phase

Initially to estimate SPR number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Water (pH 7.0) in the ratio of 60:40 % v/v. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Standard Stock Solution

Accurately weigh and transfer 10mg of SPR of working standard into 10 ml clean dry volumetric flask add about 6 ml of diluents (Mobile Phase) and sonicate to dissolve it completely and make volume up to the mark with same solvent (Stock Solution). Further pipette 1ml of above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.5 to 2.5 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. This gives the solutions of 5-25µg/ml for drug.

Preparation of Blank Plasma

To 1 ml of plasma, 4 ml of acetonitrile was added; the solution was mixed thoroughly and left to stand for 5 min at room temperature. After 5 min the solution was centrifuged at 10000 rpm for 12 min at 4°C. The clear supernatant liquid was removed, filtered through 0.22 µ syringe filter and injected directly into HPLC system.

Preparation of Standard Plasma Stock Solution

To prepare calibration standards and quality control samples, appropriate quantities of the various diluted standard solutions added to blank plasma to obtain drugs in the concentrations range of 5-25µg/ml for SPR. These were stored at –20°C and 20 µL volume of each sample was injected.

Assay in Formulations

To determine the content of the drug in the formulations, twenty marketed tablets of novospar were weighed and ground to a fine powder; amount equal to 200 mg of SPR was taken in 100ml volumetric flask before 100 ml diluents was added. The content of the flask was shaken for about 60 min. This solution was filtered through Whatman filter paper to separate out the insoluble excipients and further dilutions were carried out to obtain the desired concentration. Final solutions were filtered through a 0.45-µm Millipore filter before injection into the HPLC.
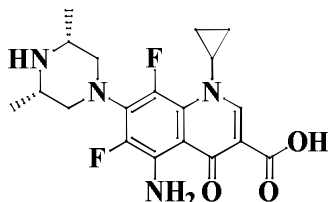

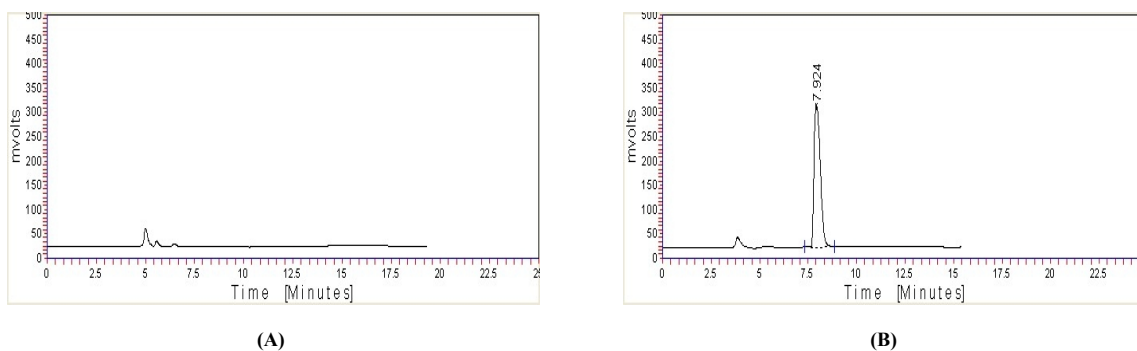
Figure 1: Chemical structure of sparfloxacin

| (A) | (B) |

Figure 2: Representative chromatogram of blank plasma (A) and sparfloxacin in formulation (B)
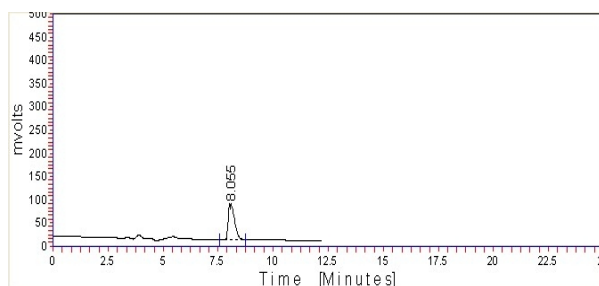
Figure 3: Representative chromatogram of sparfloxacin in human plasma

Table 1: Regression statistics and LOD and LOQ

Drug	Regression equation	*R^2	*LOD (µg/mL)	*LOQ (µg/mL)
Sparfloxacin	y = 137.1x +22.65	0.9998	0.573	1.542

*Average of five determination

Table 2: Result of precision and robustness

Validation Parameter	Percentage Mean ± S.D*. (n=15)	Percentage RSD*
Repeatability	98.64±1.28	1.29
Reproducibility	99.07±0.55	0.56
Intermediate precision		
Day to Day	98.96 ±0.73	0.74
Analyst to Analyst	98.97±0.39	0.39
Robustness*	99.28±0.46	0.47

* Mean of fifteen determinations *(3 replicates at 5 concentration level)*

Table 3: Results from recovery studies of sparfloxacin

Recovery Level %	Sparfloxacin	
	% MEAN±SD*	% RSD*
80	99.11±0.318	0.320
100	98.39±1.582	1.607
120	98.05±1.657	1.689

*Average of five determination

Table 4: Accuracy and precision in human plasma sample

Analyte	Spiked conc. (µg/mL)	Precision (% RSD)	Accuracy (%)
Sparfloxacin	10	0.92	99.60
	15	1.03	98.67
	20	1.60	97.85

*Average of five determination

Table 5: Analysis of SPR marketed formulations

Formulation	Labelled amount (mg)	Amount Found (mg)	% Assay ±SD*	% R.S.D
Novospar (200mg)	200	199.3	99.65±0.970	0.98

*Average of five determination

RESULT AND DISCUSSION

Method Validation

The method was validated according to ICH guidelines for validation of analytical procedures. The method was validated for the parameters like linearity, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and robustness. The linearity of this method was proved using linear correlation of the peak-area values and appropriate concentrations.

Linearity, Limit of Detection and Quantification

Under the previously described experimental conditions, linear correlation between the peak area and applied concentration was found in the concentration range 5-25µg/ml. The regression statistics are shown in Table 1. The LOD and LOQ at concentrations where the signal-to-noise ratios were equal to 3 and 10 respectively were determined to be 0.573µg/ml and 1.542µg/ml for SPR respectively. The correlation coefficient of this dependence was calculated to be 0.9998 for sparfloxacin.

Precision and Robustness

Precision of the methods was studied at three levels as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility in synthetic samples using placebo mixtures. Mean ± SD and % relative standard deviation (RSD) values were used to express precision. As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Methanol: Water pH 7.0 (60:40% v/v) to (55:45% v/v). Results of precision and robustness are reported in Table 2.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. The recovery studies were carried out by adding a definite concentration of standard drug (80%, 100%, and 120%) to preanalyzed sample solutions. The resulting solutions were then re-analyzed by proposed methods. The value of mean recoveries was found to be in ranging from 98.05 to 99.11 for SPR. Total amount of drug found and percentage recovery was calculated. Result of recovery studies are reported in Table 3.

Specificity

A representative chromatogram (Figure 2) was generated to show that other components, which could be present in the sample matrix, are resolved from the parent analyte. No significant changes in retention times of the drugs in the presence and the absence of excipients clearly indicated the specificity of the method.

Application in Human Plasma

It was observed after spiking the analyte in the plasma sample that there was no significant difference between the amount of drug spiked in plasma and the amount recovered. The recovery values (Table 4) in human plasma clearly indicate the applicability of the present method for the required purpose (Figure 3).

Assay of Tablet Formulation

Content of SPR found in the tablets by the proposed method are shown in Table 5. The low values of R.S.D. indicate that the method is precise, accurate and no interference of excipients in the estimation of sparfloxacin.

CONCLUSION

After studying all the results obtained by HPLC studies, it was concluded that the present method was fast and easy to perform. Moreover, the method doesn't require various elaborate treatments and tedious extraction procedures. The linearity range, precision, accuracy, robustness, LOD, LOQ and specificity were processed to establish the suitability of the method and the confirmed results were obtained. HPLC has several superiorities compared with UV spectrophotometry, such as smaller detection and quantification limits, small sample volumes and specificity. The proposed method as higher sensitivity than many of the reported methods. Thus, the developed HPLC method is green, rapid, reliable, cost-effective and so, it is inexpensive and ecofriendly. In addition to the satisfactory, sensitivity and reproducibility as well as the convenience and simplicity and can be proposed for routine analysis laboratories and quality control purposes and are very beneficial for pharmaceutical companies, clinicians and physicians and also can be beneficial for the studies of drug interaction with other combinations.

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