

## A VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF SPARFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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### ABSTRACT

**Objective:** To develop a simple, accurate, precise and selective isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method was developed for the quantitative estimation of Sparfloxacin in pharmaceutical formulations.

**Experimental:** RP-HPLC method was developed by using Welchrom C<sub>18</sub> Column with 250 mm x 4.6 mm i.d and 5µm particle size, SHIMADZU LC-20AT Prominence liquid chromatograph. The mobile phase used is phosphate buffer (pH-3.0): acetonitrile (70:30% v/v) with a flow rate of 1mL/min. The eluent was monitored by UV detection at 291nm using SHIMADZU SPD-20A prominence UV-Vis detector.

**Results:** The retention time of Sparfloxacin found to be 5.499 min. The method retains linearity in the range of 2-10µg/mL with correlation coefficient 0.9999. The mean recovery of sparfloxacin was 99.702% to 100.456%. The LOD and LOQ of the drug were found to be 0.186 µg/mL and 0.558 µg/mL respectively.

**Conclusion:** The proposed method can be successfully used for routine determination of sparfloxacin in pharmaceutical formulations.

**Keywords:** Sparfloxacin, Isocratic RP-HPLC, Correlation coefficient, Method Validation.

### INTRODUCTION:

The new fluoroquinolone Sparfloxacin (SPF) is (5-Amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[1] (Figure 1), is a broad spectrum fluorinated a quinolone antibiotic used in the treatment of bacterial infections and commonly prescribed for infective ophthalmitis and sinusitis, acute exacerbation of chronic bronchitis, community-acquired pneumonia, eye infections, urinary tract infection. SPF is a new difluorinated quinolone with similar activity for gram-negative and gram-positive bacteria and a spectrum of activity that embraces anaerobes, Chlamydia trachomatis, Mycoplasma and mycobacteria [2]. The quinolones and SPF compounds are bactericidal in nature. The molecular target of quinolones is considered to be DNA gyrase, since quinolones inhibit gyrase activities and gyrases isolated from quinolone-resistant strains are resistant to quinolones [3-4]. Escherichia coli gyrase consists of subunits A and B which are the products of the gyrA and gyrB genes, respectively [5-7] since the unexpected finding by Shen and Pernet that [3H] norfloxacin binds to DNA but not to purified gyrase [8] it has been proposed that SPF exerts its antibacterial activity by inhibiting DNA gyrase which is a bacterial topoisomerase. DNA gyrase [9] is an essential enzyme which controls DNA topology and assists in DNA replication, repair, deactivation and transcription.

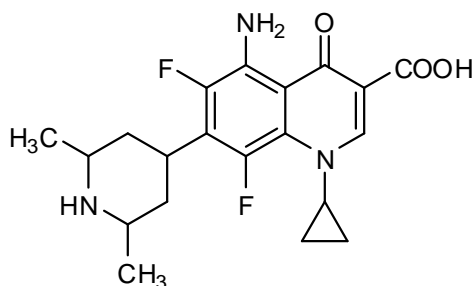


Figure 1: Structure of SPF

Literature survey revealed that very few methods have been reported for the analysis of SPF which includes luminescence spectroscopy, Reverse Phase High Pressure liquid Chromatography [10], RP-HPLC with fluorescence detection [11-18] and few UV spectrophotometric methods [19]. However most of the available RP- HPLC methods have limitations such as long run times, low sensitivity, uneconomical and have poor symmetry. Keeping in view of these we have decided to develop a simple, accurate, precise and reliable RP-HPLC method for the estimation of SPF in pharmaceutical dosage forms as per ICH guidelines [20]. The goal of this study is to develop rapid HPLC method for the analysis of SPF in bulk drug samples and tablet formulations using the most commonly employed column C<sub>18</sub> with UV detection at suitable wavelength.

### MATERIALS AND METHODS

#### Chemicals and reagents

SPF active pharmaceutical ingredient (API) was obtained from FDC Limited, Mumbai, India. HPLC grade acetonitrile, water and analytical reagent (AR) grade Potassium dihydrogen orthophosphate dehydrate and orthophosphoric acid from Merk chemicals, Mumbai, India. While Commercial tablets of SPF was acquired from local market. Sparcip100mg (Cipla), Zospar 200mg (FDC Ltd.), Mumbai, India are the commercial formulations.

#### Instruments

The HPLC analysis was performed on a high pressure isocratic high performance liquid chromatograph (SHIMADZU LC-20AT prominence liquid chromatograph) equipped with two LC-20AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength SHIMADZU SPD-20A prominence UV-Vis detector and WELCHROM C<sub>18</sub> Column with 250mm x 4.6mm i.d and 5µm particle size. The HPLC system was equipped with "Spinchrom CFR" software. Moreover an analytical balance (Shimadzu TX223L), digital pH meter (systronics model 802), a sonicator (Spectra lab, model UCB 40), UV-Visible spectrophotometer (Systronics model-2203) were used in present study.

## Method development

For developing the method a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and choice of stationary and mobile phase. The following studies were conducted for this purpose.

### Selection of analytical wave length:

The spectrum of diluted solutions of SPF in mobile phase was recorded on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the standard drug showed that the wavelength was found to be 291 nm is shown in Figure 2.

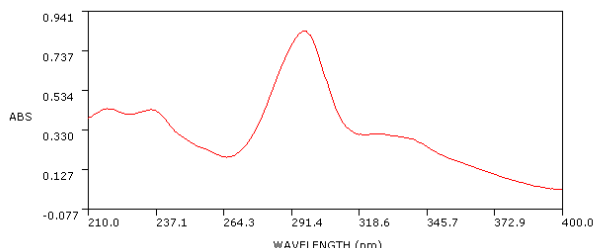


Figure 2: Absorption spectra of Sparfloxacin (291nm)

### Chromatographic conditions

Decorous selection of the stationary phase depends upon the nature of the sample, solubility and molecular weight. SPF was analyzed by reversed phase column. RP-HPLC method was developed by using Welchrom C<sub>18</sub> column with 250mm x 4.6mm i.d and 5µm particle size, SHIMADZU LC-20AT Prominence liquid chromatograph. Various combinations of buffer and acetonitrile were tested. Finally the mixture of phosphate buffer (pH-3.0): acetonitrile (70:30% v/v) was selected as a mobile phase. Composition of mobile phase on the retention time of SPF was thoroughly investigated. The concentration of the phosphate buffer pH-3: acetonitrile 70:30% v/v was optimized to give symmetric peak with short run time. UV detection wave length was 291nm, flow rate was 1mL/min and injection volume was 20 µL.

### Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 6.056 g of KH<sub>2</sub>PO<sub>4</sub> in 445 mL of HPLC grade water. To this 55mL of 0.1M phosphoric acid was added and pH was adjusted to 3.0 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 70: 30 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

### Preparation of calibration standards

100 mg of SPF was accurately weighed and dissolved in 100 mL of mobile phase to get 1 mg/mL stock solution. Working standard solution of SPF was prepared with mobile phase. To a series of 10mL volumetric flasks, standard solutions of SPF in the concentration range of 2, 4, 6, 8, and 10 µg/mL were transferred. The final volume was made with the mobile phase.

### System suitability

System suitability tests are an integral part of chromatographic method which was used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg/mL for SPF to check the reproducibility of the system. At first the HPLC system was stabilized for forty min. One blank followed by six replicates of a single calibration standard solution of SPF was injected to check the system suitability. To ascertain the systems suitability for the proposed method, a number of parameters such as retention time, theoretical plates, peak asymmetry (tailing factor) and results are presented in Table 1.

Table 1: Optimized chromatographic conditions and system suitability parameters

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT Prominence liquid chromatograph
Column	WELCHROM C <sub>18</sub> column with 250mm x 4.6mm i.d and 5µm particle size.
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Mobile phase	Buffer: ACN (70 : 30 v/v)
Flow rate	1mL/min.
Detection wave length	UV at 291nm.
Run time	10 minutes
Column back pressure	119-120 (kg/cm <sup>2</sup> )
Temperature	Ambient temperature
Volume of injection loop	20(µL)
Retention time	5.499 min
Theoretical plates[th.pl] (Efficiency)	14761
Theoretical plates per meter[t.p/m]	294245
Tailing factor	1.073

### Recommended procedure:

#### Calibration curve for SPF

Replicates of each calibration standard solutions (2, 4, 6, 8, and 10 µg/mL) were injected using a 20µL permanent loop system and the chromatograms were recorded. Calibration curves were constructed by plotting by taking concentrations of SPF on X-axis and average peak areas of standard SPF on Y-axis and regression equations were calculated for SPF. The linearity range was found to be 2-10µg/mL. Summary of regression analysis parameters for proposed method results are presented in Table 2.

Table 2: Summary of regression analysis parameters for proposed method:

Parameter	Method
Detection wavelength(λ max)	By UV at 291nm
Linearity range (µg/mL)	2-10µg/mL
Regression equation (Y=a+bx)	Y=43.07X
Slope(b)	43.07
Intercept(a)	0
Standard error of slope (S <sub>b</sub> )	0.72900
Standard error of intercept (S <sub>a</sub> )	2.42778
Standard error of estimation (Se)	1.50016
Correlation coefficient (r <sup>2</sup> )	0.9999
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.104
Limit of detection (LOD)	0.186 µg/mL
Limit of quantitation (LOQ)	0.558 µg/mL
Percentage range of errors* (Confidence limits)	
0.005significance level	0.12232
0.001 significance level	0.19183

\* Average of 6 determinations.

### Analysis of marketed formulation

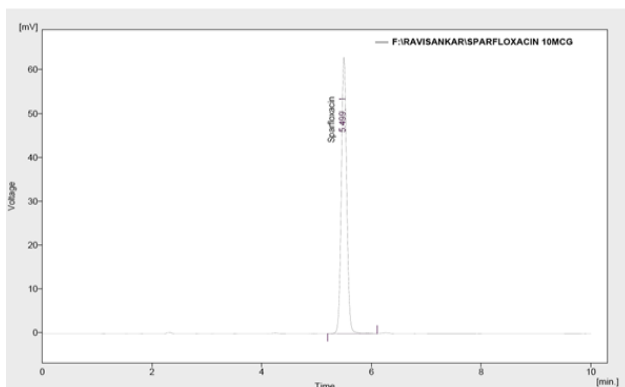
The content of twenty tablets was accurately weighed and transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 100 mg of SPF was taken and the drug was extracted in 100 mL of mobile phase. The resulting solution was filtered through 0.45µm membrane filter paper and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µL fixed volume loop manual injector. The chromatographic run time of 10 min. was upheld for the elution of the drug from the column. The effluents were monitored with UV detector at 291 nm. A 20 µ volume of sample solutions were separately injected on HPLC system. From the peak area of SPF the

amount of drug in the sample were figured. The content was calculated as an average of six determinations and experimental results are presented in Table 3. The representative standard and sample chromatograms of SPF are displayed in Figure 3 and Figure 4.

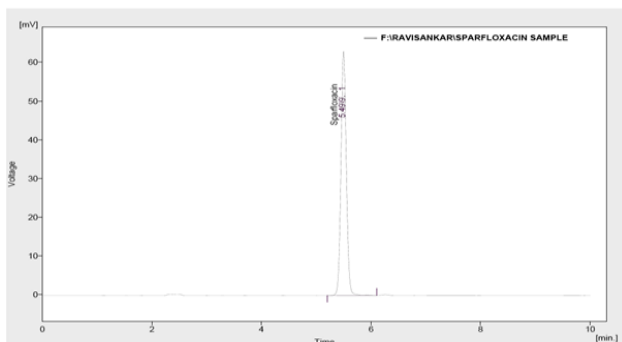
**Table 3: Analysis of SPF marketed formulations**

S.No	Formulations	Labeled amount	Amount found	% Assay
		(mg)	(mg)	±SD*
1	Sparcip100mg (Cipla)	100	99.45	99.45±0.10
2	Zospar 200mg(FDC)	200	199.2	99.60±0.098

\* Average of 6 determinations



**Figure3: A typical chromatogram of SPF standard**



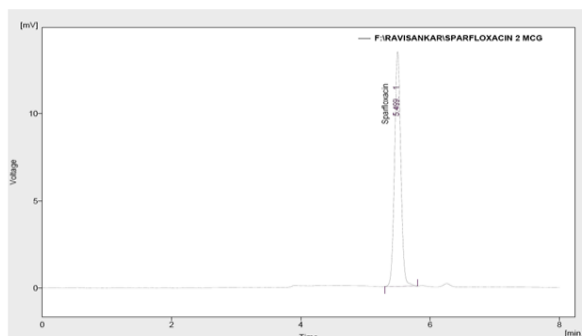
**Figure 4: Chromatogram of marketed formulation (Tablets) of SPF**

**Validation study of the proposed method**

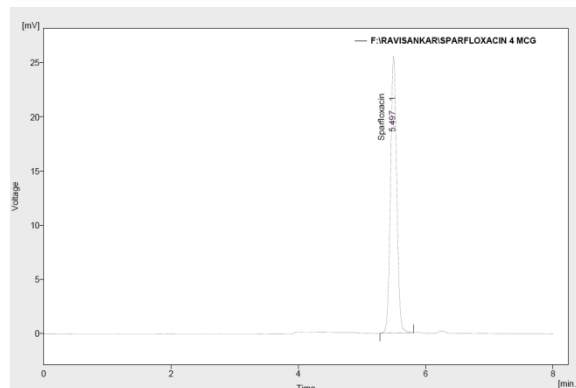
The developed method of analysis was validated as per the ICH guidelines. The parameters studied for validation were linearity, specificity, Precision, accuracy, robustness, system suitability, limit of detection (LOD) and limit of quantification (LOQ).

**Linearity**

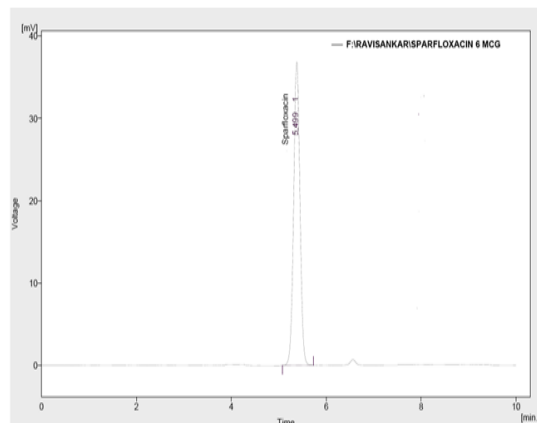
It is the ability of an assay to elicit a direct and proportional response to changes in analyte concentration. The linearity graphs for the proposed assay methods were obtained over the concentration range of 2-10 µg/mL SPF. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values and the results were presented in Table 2. The representative chromatograms indicating the SPF were shown in Figure 5 to 9. A calibration curve was plotted between concentration and peak area response and calibration curve is shown in Figure 10.



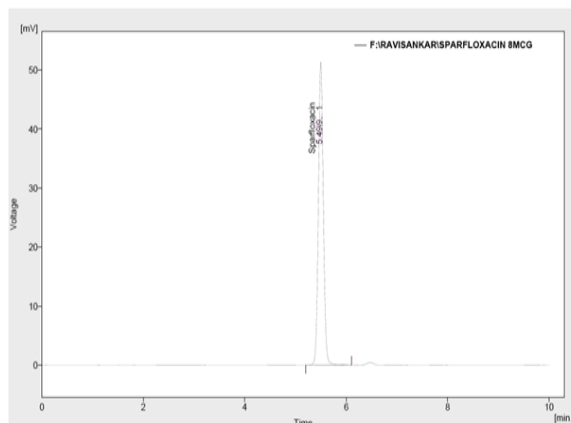
**Figure 5: Standard chromatogram of SPF (2 µg/mL)**



**Figure 6: Standard chromatogram of SPF (4 µg/mL)**



**Figure 7: Standard chromatogram of SPF (6 µg/mL)**



**Figure 8: Standard chromatogram of SPF (8 µg/mL)**

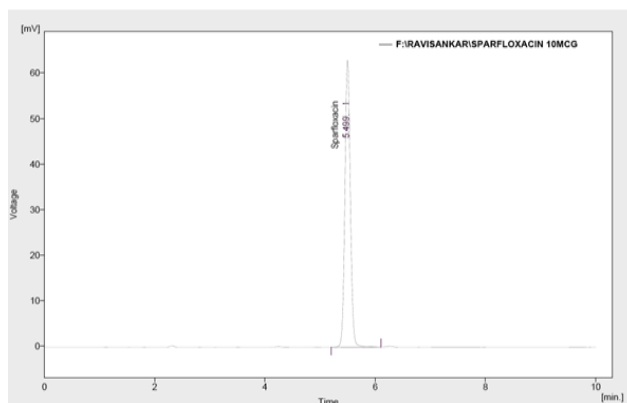


Figure 9: Standard chromatogram of SPF (10 µg/mL)

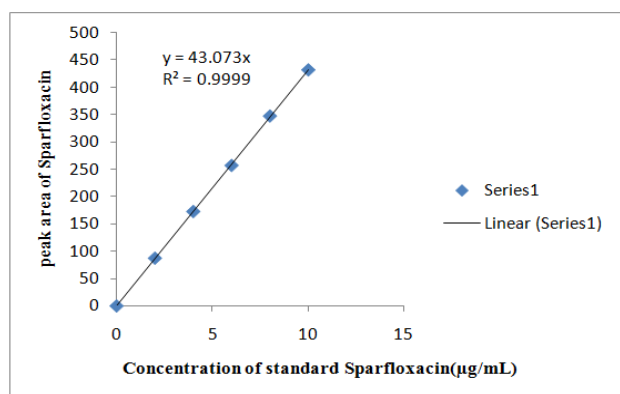


Figure 10: Calibration plot of SPF

### Specificity

Specificity is ability of an analytical method to measure the analyte free from interference due to other compounds. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the sample solution injected and tested. The specificity results are summarized in Table 4.

Table 4: Specificity study

Name of the solution	Retention time in minutes
Blank	No peaks
SPF	5.499 min.

**Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Intraday and interday precision study of SPF was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 10 µg/mL. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0 and results are presented in Table 5.

Table 5: Results of Intraday and interday precision study:

Sample	Injection number	Intraday precision	Interday precision
		Peak area	Peak area
SPF	1	431.45	431.62
	2	429	432.31
	3	431.11	430.19
	4	430.26	429.27
	5	426.7	430.44

6	430.05	428.39
Mean	429.92	429.72
Standard deviation	0.502	0.7557
% RSD	0.1145	0.1721

### Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of SPF by the method of addition. Known amount of SPF at 80%, 100%, and 120% was added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of SPF at each level was not less than 99% and not more than 101%. The % recovery was found to be within the limits as listed in Table 6.

Table 6: Recovery studies

Recovery level <sup>#</sup>	SPF	
	% Recovery* ±	SD
80%	99.702 ± 0.461	
100%	100.456 ± 0.402	
120%	100.232 ± 0.423	

<sup>#</sup> Each recovery level is tested triplicate.

\* Average of triplicate determinations

### Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was evaluated by the analysis of SPF under different experimental conditions such as making small changes in flow rate (± 0.2 mL/min), detection wavelength λ<sub>max</sub> (± 5 nm), Mobile phase composition (±5%), and pH of the buffer solution. The Robustness results are presented in Table 7.

Table 7: Results of robustness

S. No	Parameters	Optim ized	Used	Retentio n time (t <sub>r</sub> )	Peak asymmet ry
1	Flow rate (±0.2)	1 mL/min	0.8	5.525	1.091
			1	5.499	1.073
2	λ max(±5)	291nm	1.2	5.427	1.086
			286	5.498	1.059
3	Mobile phase composition (±5)	70:30:00	291	5.499	1.073
			296	5.499	1.095
			65:35:00	5.477	1.187
3	Mobile phase composition (±5)	70:30:00	0	5.499	1.073
			75:25:00	5.523	1.172

### LOD and LOQ

LOD is the lowest concentration in a sample that can be detected but not necessarily quantified. Under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantitation were calculated using following formula LOD=3.3s/S and LOQ= 10s/S, where s= standard deviation of blank readings or standard deviation of regression line and S= Slope of the calibration curve. The results are presented in Table 8.

Table 8: LOD and LOQ

Limit of Detection(LOD)	0.186 µg/mL
Limit of Quantitation(LOQ)	0.558 µg/mL

## RESULTS AND DISCUSSION

The mobile phase comprising of phosphate buffer (PH-3.0): acetonitrile (70:30% v/v) at 1mL/min flow rate was optimized which gave sharp peak, minimum tailing factor with short runtime for SPF. The retention time for SPF was 5.499 min. UV spectra of SPF exhibited that the drug absorbed maximum at 291 nm, so this wavelength was carefully chosen as the detection wavelength. System suitability parameters were very satisfactory. The proposed method was found to be linear over the range of 2-10µg/mL. The regression equation was found to be  $Y=43.07 x$  with correlation coefficient ( $R^2$ ) value of 0.9999, which states that the method was good linear to the concentration versus peak area response. The developed method was applied to the assay of SPF tablets. The results were very close to labeled value of commercial tablets. The proposed method was found to be specific for SPF drug and no interferences were found at the retention time of the SPF peak and furthermore the well-shaped peaks also indicate the specificity of the method. The Precision was studied to find out intra and inter day variations in the test methods of SPF for the three times on the same day and different day. The intra-day and inter-day precision obtained was % RSD (< 2) indicates that the proposed method is quite precise and reproducible. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. A known amount of SPF standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits. Generally the mean percentage recovery of SPF at each level was not less than 99% and not more than 101%. In this case percentage recovery of SPF was found to be in the range of 99.702 to 100.456%. The method precision was done and the low % RSD (0.145) values indicates that the proposed method which was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, lambda max, mobile phase composition etc.; it was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limit which indicates that the method has robustness and suitable for routine use. The limit of detection (LOD) was 0.186 µg/mL and the limit of quantitation (LOQ) was 0.558 µg/mL which shows that this method is very sensitive and these parameters were found within the limits.

## CONCLUSION

A simple, specific, accurate, precise and new validated RP-HPLC method has been developed and validated for the quantitative determination of SPF in tablet dosage form in bulk and pharmaceutical dosage forms. Statistical analysis of the results shows that the proposed method has good precision and accuracy. The accuracy and precision results indicates the high quality of the method. The robustness results indicate the vast applicability of the method. The method was completely free from interference of the other active ingredients and additives used in the formulation. As a matter of fact the results of the study point out that the developed method found to be reliable, linear, sensitive, economical and reproducible. It was validated as per ICH guidelines and also satisfactory results were obtained for all validation parameters. The developed method was successfully applied to marketed dosage form and the results were found with higher confidence. Hence it can be concluded that the developed method can be successfully applied for routine quality control analysis of SPF in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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