*Section A-Review paper*

# Sparfloxacin – Analytical Method Development & Validation

**Pawal Suvarna Pradip, Ghawate Dipti Popat, Jadhav Rutuja Pravinkumar, Dr. Amit Kasabe**

**ABSTRACT**

A modified simple, selective, rapid, precise high performance liquid chromatography method has been developed and validated for the estimation of sparfloxacin. The separation was made in a HiQSil C-18 column using aqueous solution of 1% acetic acid and acetonitrile (75:25, v/v) as mobile phase at 290 nm using JASCO UV- 4075UV-VIS Detector and ChromNAV CFR chromatography software ( version 2.0). The mobile-phase flow rate and the sample volume injected were 1.0 ml/min and 20 µl, respectively. Retention time of sparfloxacin was found to be 2.28minutes respectively. The correlation coefficient of sparfloxacin was found to be 0.9996. The method was validated according to ICH guidelines with respect to linearity, accuracy, precision, robustness, specificity, etc. The developed method can be used for routine analysis of sparfloxacin .

**KEYWORDS**Sparfloxacin , HPLC , Method Development , Chromatogram , Correlation Coefficient , Method Validation .

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## 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 quinolone antibiotic used to treat bacterial infections and frequently prescribed for infective sinusitis, acute exacerbation of chronic bronchitis, community-acquired pneumonia, eye infections, and urinary tract infections. A new difluorinated quinolone called SPF has similar activity for gram-positive and gram-negative bacteria and a spectrum of activity that includes anaerobes, Chlamydia trachomatis, Mycoplasma, and mycobacteria[2]. Both quinolones and spf substances have bactericidal properties. Since quinolones inhibit gyrase activity and gyrases isolated from quinolone-resistant strains are resistant to quinolones [3–4], it is believed that DNA gyrase is the molecular

target of quinolones. Subunits A and B, which come from the gyra and gyrb genes, respectively, make up the Escherichia coli gyrase [5-7]. Since Shen and Pernet's surprising discovery that [3H] norfloxacin binds to DNA but not to purified gyrase [8], it has been hypothesized that SPF exerts its antibacterial activity by inhibiting DNA gyrase, a bacterial topoisomerase. DNA gyrase [9] is a crucial enzyme that regulates DNA topology and helps with DNA replication, repair, deactivation, and transcription.

The therapeutic activity of sparfloxacin has been researched. In the literature, there are, however, not many reports about its analytical preparation. Both as a raw substance and in tablet form, high performance liquid chromatography of sparfloxacin was described [10]. The literature has described analytical techniques for determining sparfloxacin, such as UV spectrophotometry [11] and microbiological assay [12].

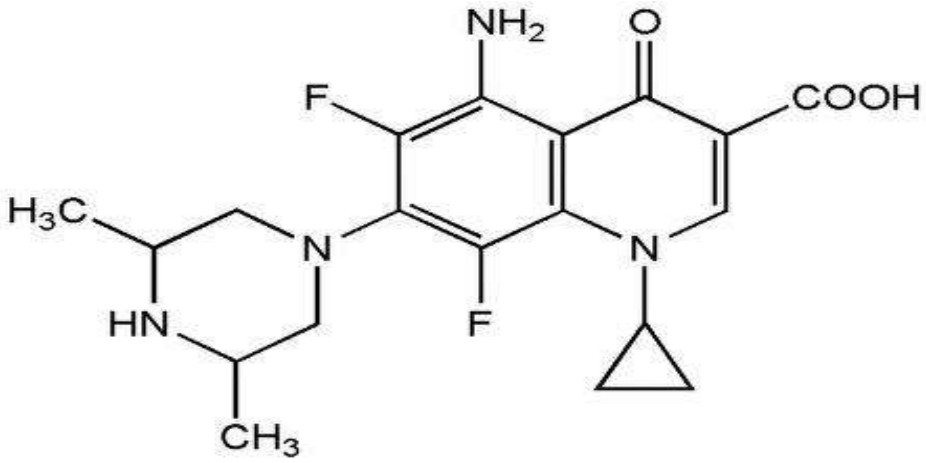


Figure 1: structure of sparfloxacin

According to a review of the literature, only a small number of techniques have been reported for the analysis of SPF, including a few UV spectrophotometric techniques [13], RP-HPLC with fluorescence detection, and luminescence spectroscopy [14-21]. This study aims to develop a quick HPLC method for the analysis of SPF using the most popular column, C18, with appropriate UV detection (22).

## ANALYTICAL METHOD DEVELOPMENT

### Sparfloxacin API

### Determination of Lambda maximum

### Preparation of stock solution of Sparfloxacin

Sparfloxacin (50mg) in a 50mL volumetric flask and 50 mL of methanol to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000

µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking methanol as blank.

The lambda maximum for Sparfloxacin was found to be 290nm.

## Instrumentation and Chromatographic Conditions

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Aqueous solution of 1% acetic acid and acetonitrile (75:25 v/v) as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 290nm. All weighing were done on Shimadzu balance (Model AY-120)

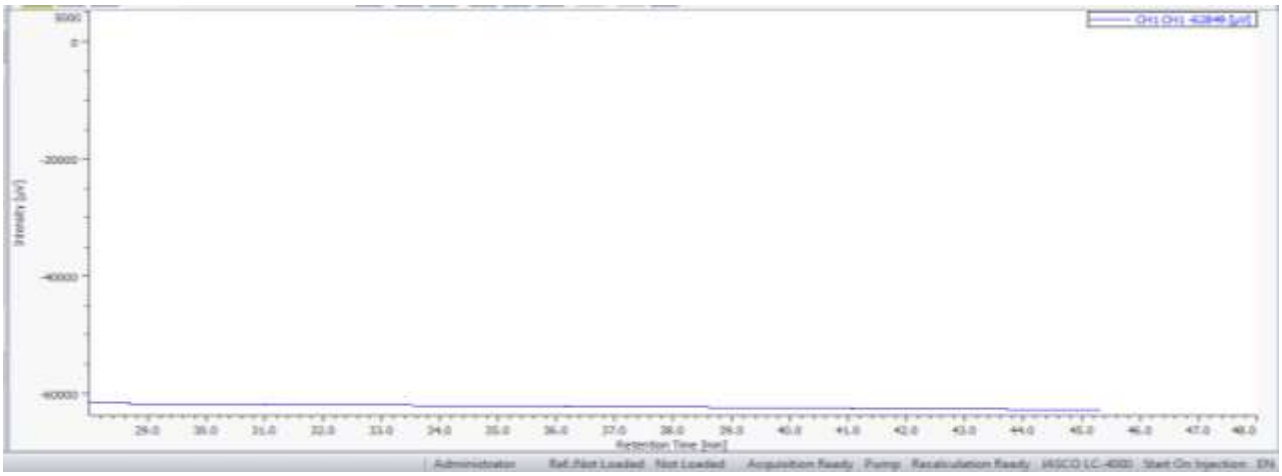

**Figure . HPLC chromatogram of blank.**

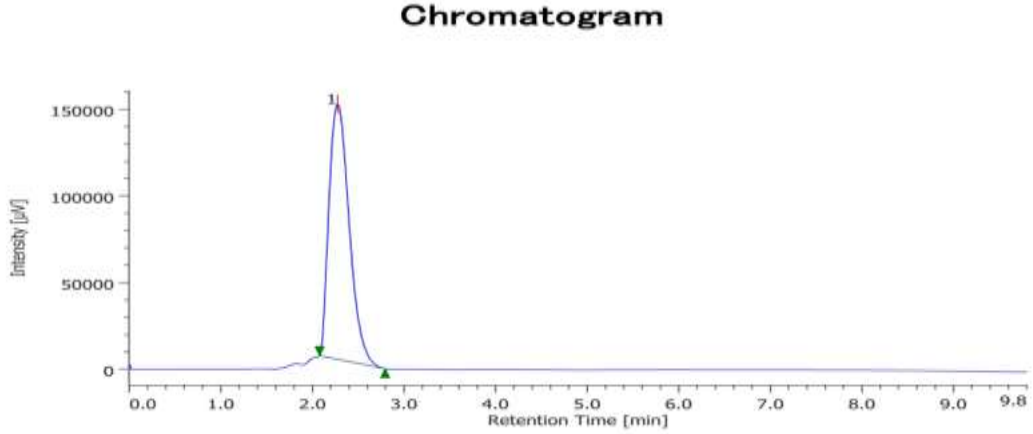

**Figure . HPLC chromatogram of standard Sparfloxacin.**

The retention time was found to be 2.28 with distinct peak.

## MATERIALS AND METHODS

### Material

Sparfloxacin standard is procured as a gift sample from Yarrow Chem Products, Mumbai (India). Chemicals utilized for method development are of HPLC grade acetonitrile, Acetic acid purchased from Merck (India) Ltd.

### Preparation of mobile phase

The preparation of mobile phase was done by mixing Aqueous solution of 1% acetic acid and acetonitrile in the ratio of 75:25. Removal of gases was carried out in ultrasonic water bath for 15 minutes. Filtered the solution through 0.45µ filter.

### Diluent preparation

Mobile phase used as diluents.

### Preparation of standard stock solution

50mg of Sparfloxacin standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase to get 1000 µg/mL. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

### Preparation of test solution

50 mg equivalent of Sparfloxacin API standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase 1000 µg/mL. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

### Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Sparfloxacin was carried out under UV ranging from 200-400nm using the standard solution.

### Method Validation

**Linearity:**

The linearity of the developed method was studied over the concentration ranges between 5-30µg/ml. The aliquots of 5, 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.5, 1, 1.5, 2.0, 2.5 and 3.0 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Sparfloxacin was constructed by plotting peak area versus used concentration of Sparfloxacin. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

**Accuracy**

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Sparfloxacin, a known amount of standard drug powder of Sparfloxacin was added to 80, 100, 120% level.

**Precision method**

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution were made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

**Limit of Detection and Limit of Quantitation**

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

**Robustness**

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

**RESULTS AND DISCUSSION**

**Selection of wavelength maxima**

The solution of Sparfloxacin was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 290nm.

**Method development**

The proposed chromatographic method was found to be suitable for effective separation of Sparfloxacin with good resolution, peak shape given in the figure. The mobile phase composed of Aqueous solution of 1% acetic acid and acetonitrile in ratio of 75:25 % v/v, at a flow rate of 1.0 ml/min was selected as it gave well
resolved peaks of standard Sparfloxacin. The optimum wavelength 290nm selected for detection and quantitation.

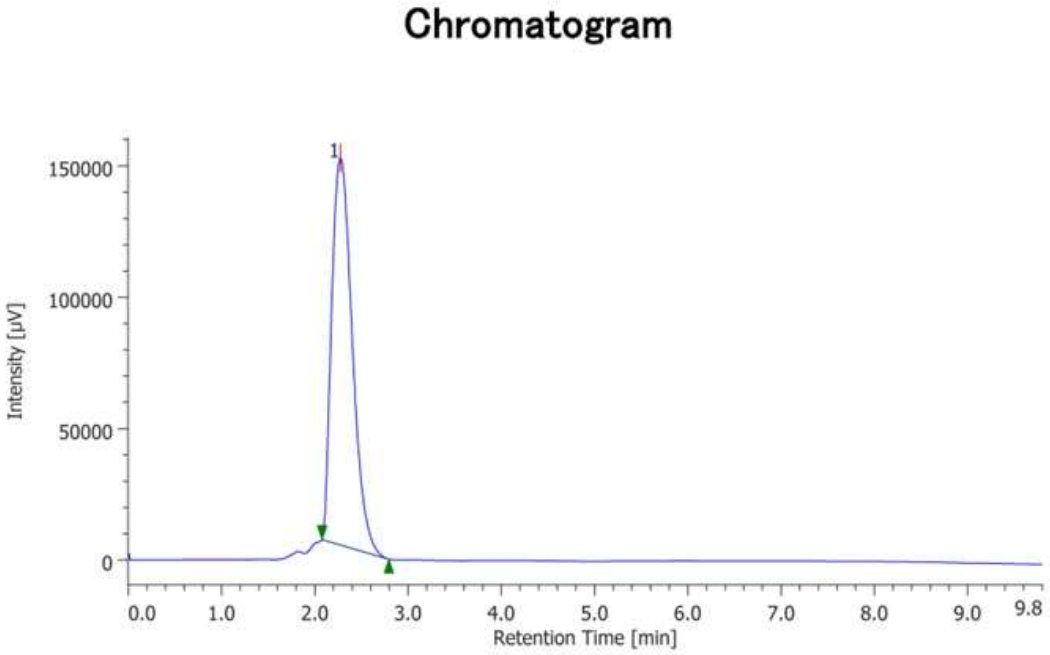


**Figure:** HPLC Chromatogram with resolved peak of Sparfloxacin

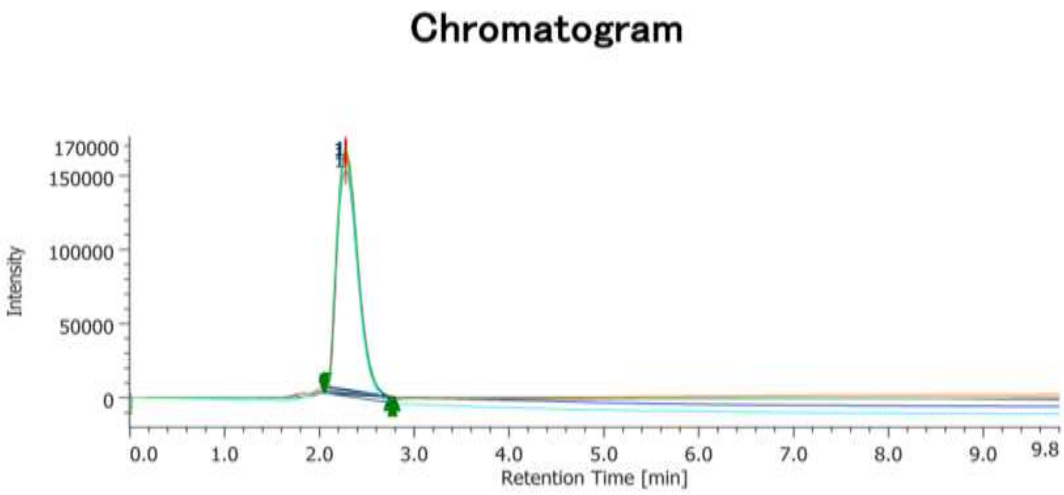


**Figure:** HPLC Chromatogram with resolved peak of Sparfloxacin (Overlay)

**Method validation**

**Linearity**

The calibration curves were found be linear for the concentration range of 5-30ppm. The standard working curve equation for drug was found to be y = 2018.3x - 154950 with correlation coefficient value $r^2$ = 0.9996. The results of linearity are given in the table and Figure.

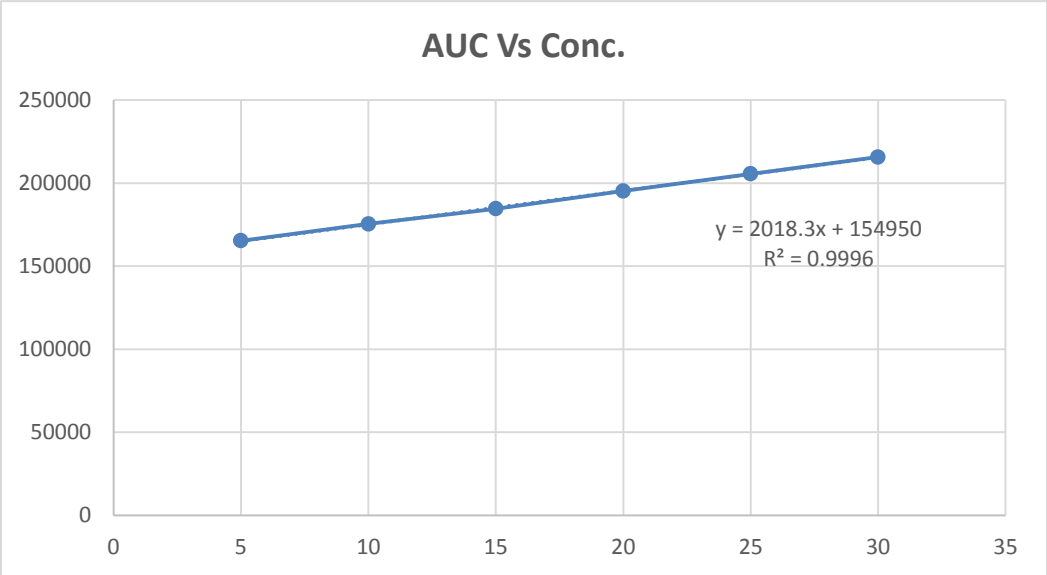


**Figure-3:** Linearity curve of standard Sparfloxacin

**Table- 1:** Linearity data of Sparfloxacin

| Concentration µg/mL | Area |
|---|---|
| 5 | 165245 |
| 10 | 175424 |
| 15 | 184526 |
| 20 | 195214 |
| 25 | 205524 |
| 30 | 215689 |

**Recovery studies**

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Sparfloxacin given in Table. The % recovery at 80, 100, and 120 % was found to be given in below table.

**Table-2:** Recovery data of Sparfloxacin

| Level (%) | Drug Conc. (mg) | Amt. recovered (mg) | % Recovery |
|---|---|---|---|
| **80%** | 8 | 8.01 | 100.12 |
| **100%** | 10 | 9.99 | 99.9 |
| **120%** | 12 | 12.54 | 104.5 |

**Precision**

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The results of precision studies are shown in Table.

**Table- 3:** Precision study (intra- day) of Sparfloxacin

| Conc. µg/mL | Area | AVG | %RSD |
|---|---|---|---|
| 10 | 175485 | 175187 | 0.29660825 |
| | 174587 | | |
| | 175489 | | |
| 15 | 185471 | 184876 | 0.27871944 |

| | | | |
|---|---|---|---|
| | 184578 | | |
| | 184579 | | |
| 20 | 195623 | 196486 | 0.4772941 |
| | 196351 | | |

Conc, Concentration; AVG, average; RSD, Relative standard deviation

**Table- 4:** Precision study (inter-day) of Sparfloxacin

| **Conc µg/mL** | **Area** | **AVG** | **%RSD** |
|---|---|---|---|
| 10 | 174512 | 174895.33 | 0.39307399 |
| | 175689 | | |
| | 174485 | | |
| 15 | 185474 | 186219.67 | 0.6264347 |
| | 185621 | | |
| | 187564 | | |
| 20 | 196325 | 196161.33 | 0.07959878 |
| | 196014 | | |
| | 196145 | | |

Conc, Concentration; AVG, average; RSD, Relative standard deviation

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

This data showed that the sensitivity of method to determine the drug Sparfloxacin. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.54 & 2.68 µg/m/ respectively.

**Robustness**

Robustness of method was measured by multiple injections of a homogenous sample containing Sparfloxacin by changing flow rate 0.8 mL/min and 1.2 mL/min, mobile phase composition Acetic acid: ACN ratio 74:26 and 76:24, wavelength i.e 289nm and 291nm. The method was found to be robust in the range of deliberate changes made.

**Table-5:** Robustness study with change in flow rate of Sparfloxacin

| **Flow rate mL/min** | **Conc. µg/mL** | **Area** | **AVG** | **%RSD** |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 0.8 | 20 | 194751 | 194792.7 | 0.03661 |
| 0.8 | | 194875 | | |
| 0.8 | | 194752 | | |
| 1.2 | 20 | 195612 | 195552 | 0.06221 |
| 1.2 | | 195412 | | |
| 1.2 | | 195632 | | |

Conc, Concentration; AVG, average; RSD, Relative standard deviation

**Table-6:** Robustness study with change in concentration of mobile phase of Sparfloxacin

| **Mobile phase** | **Conc µg/mL** | **Area** | **AVG** | **%RSD** |
|---|---|---|---|---|
| 74:26 | 20 | 196548 | 196609.3 | 0.41693 |
| 74:26 | | 197458 | | |
| 74:26 | | 195822 | | |
| 76:24 | 20 | 194751 | 196187.7 | 0.6938 |
| 76:24 | | 196354 | | |
| 76:24 | | 197458 | | |

Conc, Concentration; AVG, average; RSD, Relative standard deviation

**Table-7:** Robustness study with change in Wavelength of Sparfloxacin**.**

| **Wavelength nm** | **Conc. µg/mL** | **Area** | **AVG** | **%RSD** |
|---|---|---|---|---|
| 289 | 20 | 197452 | 196993.3 | 0.97989 |
| 289 | | 194875 | | |
| 289 | | 198653 | | |
| 291 | 20 | 190254 | 190578 | 0.24955 |
| 291 | | 190356 | | |
| 291 | | 191124 | | |

## RESULT AND DISCUSSION

The mobile phase was done by mixing aqueous solution of 1% acetic acid and acetonitrile (75:25% 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 2.28 min. UV spectra of SPF exhibited that the drug absorbed maximum at 290 nm, so this wavelength was carefully chosen as the detection wavelength. System suitability parameters were very satisfactory. correlation coefficient (R2) value of 0.9996.which states that the method was good linear . 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. Generally the mean percentage recovery of SPF at each level was not less than 99% and not more than 104%. In this case percentage recovery of SPF was found to be in the range of 99.9 to 104.5%. 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 1.54 ug/mL and the limit of quantitation LOQ was 2.68 ug/mL which shows that this method is very sensitive and there parameters were found within the limits.

## CONCLUSION

A simple, specific, accurate, precise and new validated HPLC method has been developed and validated for the quantitative determination of SPF. 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. 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. 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).

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