# Method Development and Validation for the Estimation of Sparfloxacin by RP-HPLC

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**Abstract- Sparfloxacin is 5-amino-1-cyclopropyl-7-[(3R, 5S)-3, 5-dimethylpiperazine-1-yl]-6, 8-difluro4-oxoquinoline-3-carboxyic acid. A rapid, specific and economic UV Spectrophotometric & RP-HPLC method has been developed using as a solvent dist. Water and methanol (40:60) to determination the sparfloxacin content in bulk and pharmaceutical dosage formulations. The quantitative determination of the drug has been carried out at the predetermined wavelength at 293nm, it was proved linear in the range 2-10 µg/ml and exhibited good correlation coefficient ($R^2$ - 0.999) and excellent mean recovery (98-100.9%) and simple and accurate reversed phase liquid chromatography method (RP-HPLC) method was developed for the quantitative estimation of sparfloxacin.The drug was chromatographic on a reversed phase C-18 column. Eluents were monitored at a wavelength of 298 nm using a mixture of methanol:0.1%TFA (25:75). The retention time of sparfloxacin was found to be 4.10 minutes. The flow rate of the mobile phase was 1.0 ml/minat room temperature. The %recovery lies range of 99.98%.**

**Keywords- Sparfloxacin, ICH, UV spectroscopy, HPLC, FT-IR, SOP**

## I.INTRODUCTION

Sparfloxacin, 5-amino-1-cyclopropyl-7-(cis-3, 5-dimethyl-1-piperazinyl)-6, 8-difluro-1, 4 –dihydro-4-oxo-3-quinolinecarboxylic acid is a difluroquinolone antibacterial agent. The drug is not official in any pharmacopeia, hence no official method is available for the estimation of the drug in formulation.[1] It is potent fluoroquinolone antibacterial agent against gram-negative and positive bacteria. [2]

Sparfloxacin is a long-acting broad spectrum synthetic antibacterial agent belongs to the class fluoroquinolone. It is used for treatment of bacterial infections like other quinolones. It is DNA-gyrase and topoisomerase-4 inhibitor used for treatment of urinary tract infection and respiratory tract infection.[3]Quinolone interfere with DNA replication and transcription, and are bactericidal against most bacterial strains.[4]fluoroquinolone have been the primary agents for treating urinary tract infection and infections of the digestive tract and respiratory system.[5]

Analytical techniques are developed and validated for active pharmaceutical ingredients, excipients, drug products, degradation products and related substance, residual solvents etc. as a result it has become an integral parts of the requirement of the regulatory authorisation.[6]Pharmaceutical Analysis is used to determining the qualitative and quantitative composition of material under study.[7]

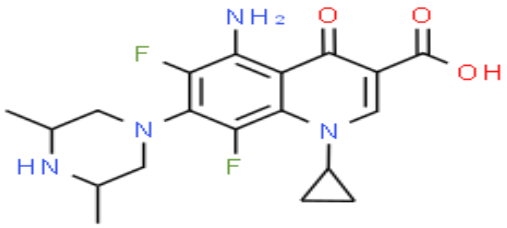


Fig. 1 sparfloxacin structure

Pharmaceutical analysis plays a very important role in quality assurance and quality control of bulk drugs and pharmaceutical dosage formulation. Rapid increase in pharmaceutical industry and production of drugs in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. [8, 9]

## II.MATERIALS AND METHODS

Reagent and chemicals:

Trifluroacetic acid, methanol, distilled water, and acetonitrile of HPLC grade were purchased from Mark Ltd., Mumbai. Standard sparfloxacin was proceeding from Rakshit pharmaceutical ltd., Mumbai. Tablet of brand Zospar tablet having a label claim 200 mg sparfloxacin was used. Water of HPLC grade obtained from UV water.

Equipment's:

| | |
|---|---|
| Electronic balance | Phoenix ISO 9001 company |
| pH meter | LABINDIA |
| Ultrasonicator | Ultrasonic Pci analytical sonicator |
| UV-Visible spectrometer | Schimadzu model UV-1800 |
| FT-IR | PerkinElmer FT-IR |
| HPLC | Agilent 1260 infinity System |

Table.1 equipment

Agilent column

UV condition:

Mobile phase as diluents: methanol: water (60:40)

HPLC condition:

A kinetex XB-C-18(150 x 4.6mm, 5µ).

Diluent: 0.1%TFA: Methanol (50:50)

Mobile phase consisting mixture of 0.1%TFA and methanol (75:25) was pumped at 1ml/min.

Materials requirements

Sample preparation forUV:

Preparation of standard stock solution-1 for UV Spectroscopy:

Initially prepare a standard stock solution of sparfloxacin by adding 10mg in 10ml volumetric flask and add 5ml diluent and mix and sonicate for 5 min. make up the volume to 10ml with MP. (60:40) (conc. = 1000µg/ml). Pipette out 1 ml of SSS-1 in 10 ml volumetric flask. Add 5 ml diluent and vortex make up the volume with diluents (conc. 100µg/ml).

Preparation of sample:

10 tablets were weighed and average tablet weight was calculated. 10 mg sparfloxacin is equivalent to 18.62mg weighed and dissolved in 10 ml of methanol: water (60:40), 1000µg/ml solution was prepared. Pipette out 1ml and make its volume up to 10 ml by diluentsin 10ml volumetric flask (conc. = 100µg/ml).

Preparation of calibration curve:

Calibration curve for sparfloxacin by UV and HPLC of different concentrations of standard sparfloxacin ranging from 2-10 µg/ml and 40-60 µg/ml respectively. The solution was prepared for UV and HPLC by pipette out 0.2, 0.4, 0.6, 0.8 and 1 mlfrom 100µg/ml and 0.8ml, 0.9ml, 1ml, 1.1ml, 1.2ml from 50µg/ml solution of sparfloxacin make up to 10 ml volumetric flask respectively.

Selection of analytical wavelength:

Appropriate dilutions were prepared for sparfloxacin from standard stock solution. The solution were scanned in the wavelength range from 200-400nm. The standard peak of sparfloxacin was found to be 293nm (UV) against methanol and water (60:40) and 298 nm (HPLC) against methanol and 0.1% TFA as blank.

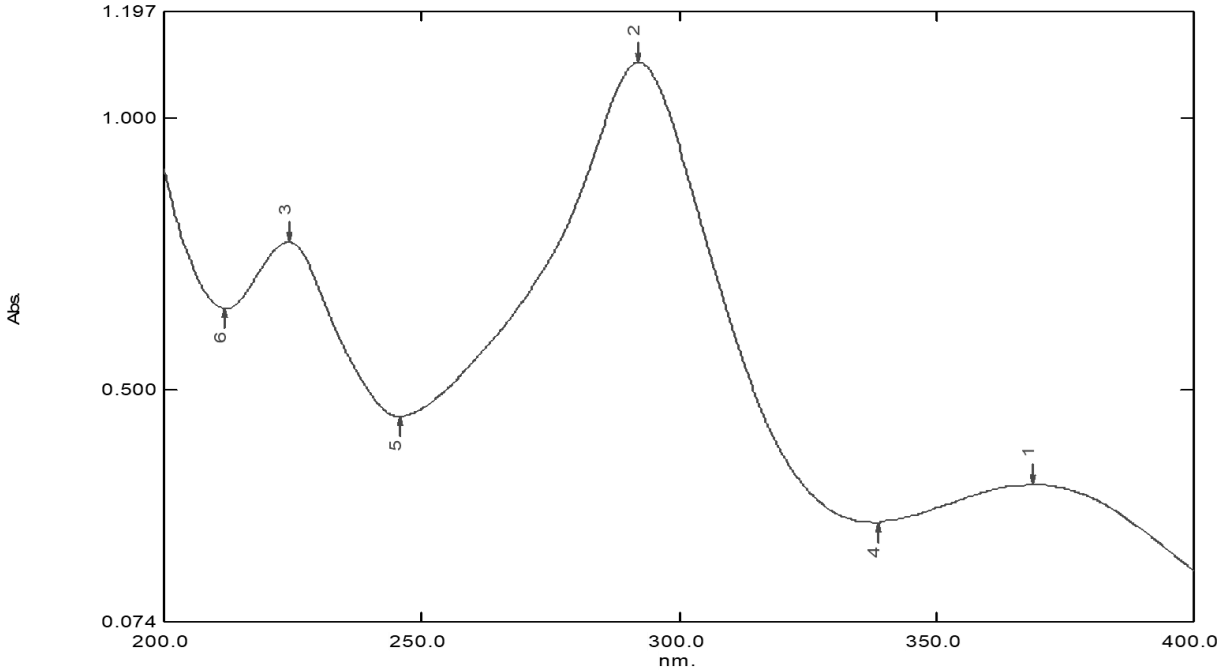


Fig.2 UV spectra of sparfloxacin

Sample preparation for HPLC

Preparation of standard stock solution for HPLC:
Initially prepare a standard stock solution of sparfloxacin by adding 5 mg in 10 ml volumetric flask and add 5 ml diluent and mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (conc. = 500µg/ml). Pipette out 1 ml of SSS-2 in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (conc. = 50µg/ml)

Preparation of sample for HPLC:
10 tablets were weighed and average tablet weight was calculated. These tablets were powdered in mortar and pestle. Powder equivalent to 5 mg sparfloxacin, was weighed and dissolved in 10 ml diluent (500µg/ml). Pipette out 1 ml of tablet stock solution in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (conc. =50µg/ml)
So, 9.69 mg of powder was dissolved in 10 ml diluent in volumetric flask to prepare tablet stock solution. (conc. =500µg/ml), pipette out 1 ml of tablet stock solution in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (50µg/ml)

Preparation of 0.1% TFA:
In a 1000ml measuring cylinder, take 700ml of HPLC Grade water, add 1ml of trifluroacetic acid and mix well, make up to the mark with HPLC grade water. Filter twice using 0.45 µ nylon filter membrane.

Chromatographic conditions:
Mobile phase :methanol: 0.1 %TFA (25:75)

Column : PhenomenexKinetex XB-C18 (150x4.6mm,5µ)

Detection wavelength:298nm

Injection volume :10µL

Flow rate :1ml/min

Retention time :4.10min

Diluent :methanol: 0.1%TFA (50:50)

Method validation:
The developed method was validated as per ICH guidelines in term of parameter like linearity, range, specificity, accuracy, precision, LOD and LOQ, robustness.

Linearity:

| Sr. No | Concentration (UV) | Concentration (HPLC) | Volume taken from above standard stock solution-1 | Volume taken from above standard stock solution-2 | Makeup volume with diluents up to |
|---|---|---|---|---|---|
| 1 | 2 | 40 | 0.2 | 0.8 | 10ml |
| 2 | 4 | 45 | 0.4 | 0.9 | 10ml |
| 3 | 6 | 50 | 0.6 | 1 | 10ml |
| 4 | 8 | 55 | 0.8 | 1.1 | 10ml |
| 5 | 10 | 60 | 10 | 1.2 | 10ml |

Table.2 linearity

## III.RESULTS

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 solution of sparfloxacin at five different concentrations. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed in fig.3 and 4.

| Parameters | UV | HPLC |
|---|---|---|
| Range | 2-10µg/ml | 40-60µg/ml |
| Regression equation | Y= 0.1055x+ 0.0559 | Y= 49784x+67645 |
| Slope | 0.1055 | 49784 |
| Intercept | 0.0559 | 67645 |
| Coefficient correlation | 0.995 | 0.997 |
| LOD | 0.020 | 4.77 |
| LOQ | 0.021 | 14.47 |

Table.3 linear regression data for UV

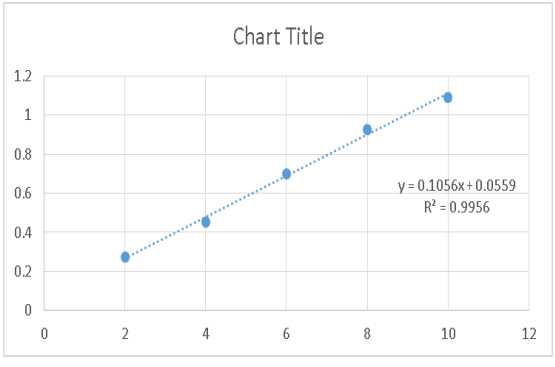


Fig.3 Calibration curve on UV

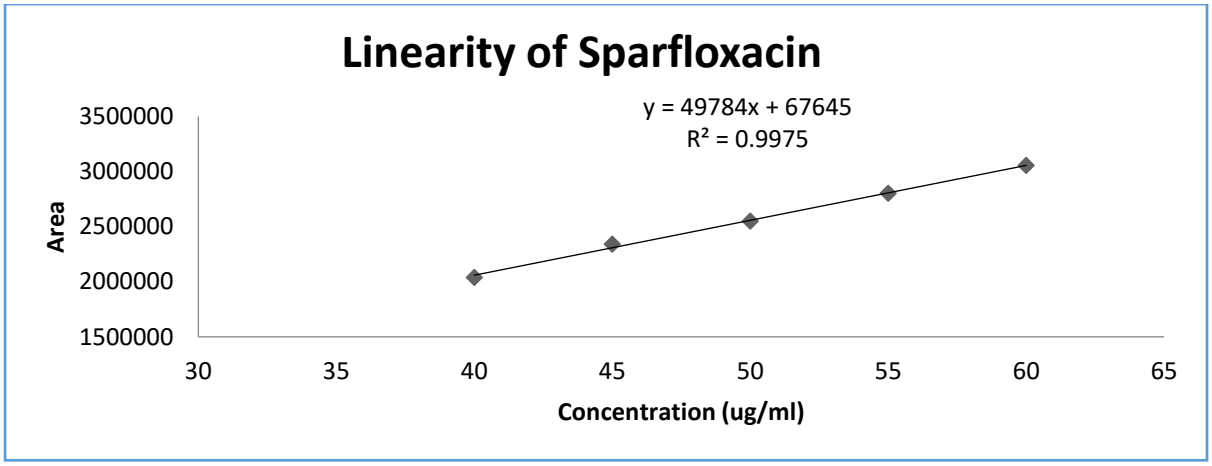


Fig.4 Calibration curve on HPLC

System suitability:

System suitability test were carried out on freshly prepared solution of reference standard of sparfloxacin and a sample was prepared as described and 6 injections were made from same sample and checked for system suitability. To check various parameters like retention time, tailing factor, theoretical plate etc.

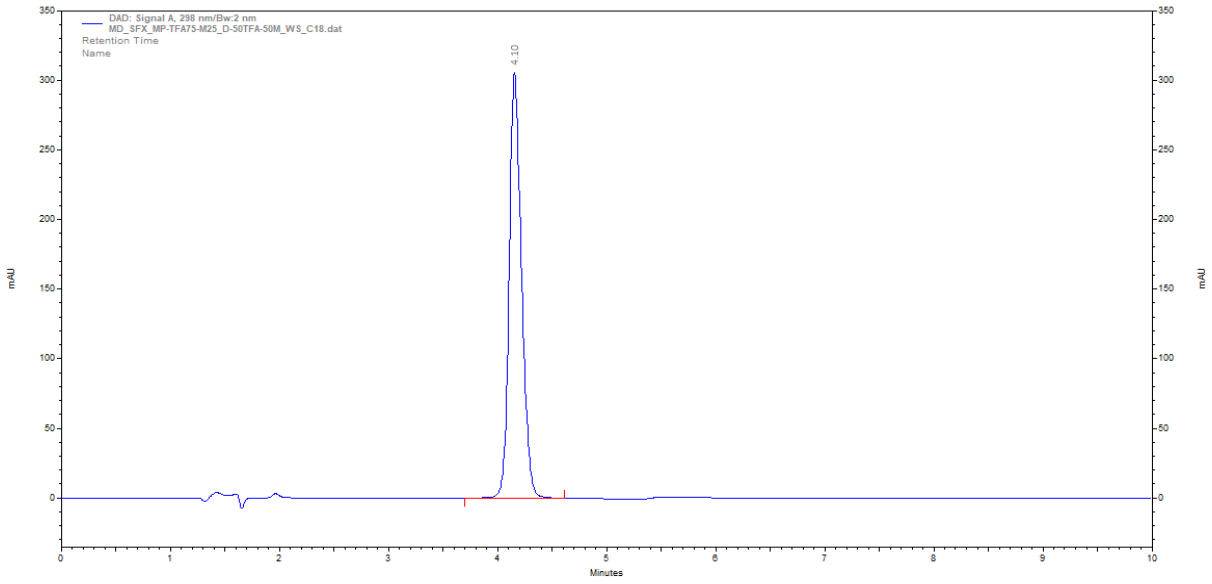


Fig.5 optimized chromatogram of sparfloxacin

| Parameters | RT | Theoretical plate | Asymmetric factor | Average | RSD |
|---|---|---|---|---|---|
| Factor | 4.10 min | 11774 | 1.07 | 4.10 | 0.77 |

Table.4 system suitability by HPLC

Precision:

The precision of an analytical method in the closeness of replicate results obtained from analysis of homogenous sample.

| Sample ID | Concentration | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | SD | RSD |
|---|---|---|---|---|---|---|---|---|
| Intra-day | 2µg/ml | 0.278 | 0.277 | 0.275 | 0.280 | 0.289 | 0.00549 | 1.95 |
| | 6 µg/ml | 0.71 | 0.707 | 0.711 | 0.705 | 0.719 | 0.0061 | 0.86 |
| | 10µg/ml | 1.086 | 1.088 | 1.09 | 1.08 | 1.088 | 0.0034 | 0.31 |
| Inter-day | 2µg/ml | 0.273 | 0.278 | 0.275 | 0.276 | 0.278 | 0.00225 | 0.81 |
| | 6 µg/ml | 0.701 | 0.708 | 0.704 | 0.705 | 0.702 | 0.0031 | 0.45 |
| | 10µg/ml | 1.091 | 1.092 | 1.09 | 1.093 | 1.094 | 0.0014 | 0.13 |
| Repeatability by HPLC | 10µg/ml | 254927 | 2541214 | 2564211 | 2571441 | 2554108 | 192576.9 | 0.77 |

Table 5 precision data

Accuracy:

The accuracy of an analytical method is closeness of results obtained by that method to be true value for the sample. To evaluate accuracy of the proposed method, recovery test were carried out. Recovery tests were performed by adding known amount of standard solution (80,100 and 120%) to the preanalyzed sample. The recoveries studied were performed in triplicate and the % recoveries were calculated. In table.6and 7, the lowest value of %RSD of assay indicate the method is accurate and there is no interference from excipients.

| Accuracy level | Absorbance | % recovery | SD | %RSD |
|---|---|---|---|---|
| 80% | 0.256 | 99.07 | 0.145 | 0.8149 |
| | 0.254 | | | |
| | 0.253 | | | |
| 100% | 0.277 | 98.20 | 0.305 | 1.5571 |
| | 0.271 | | | |
| | 0.272 | | | |
| 120% | 0.302 | 101.1 | 0.109 | 0.4897 |
| | 0.304 | | | |
| | 0.302 | | | |

Table.6 accuracy data for UV

| Accuracy level | Amount spiked | Area | Amount recovered | %recovery |
|---|---|---|---|---|
| 80 % | 39.88 | 2039502 | 39.81 | 99.75 |
| | 39.88 | 2038256 | 39.78 | |
| | 39.88 | 2039851 | 39.81 | |
| 100 % | 49.85 | 2549297 | 49.76 | 99.81 |
| | 49.85 | 2541214 | 49.60 | |
| | 49.85 | 2564211 | 50.05 | |
| 120% | 59.82 | 3053925 | 59.61 | 100.12 |
| | 59.82 | 3068541 | 59.89 | |
| | 59.82 | 3059845 | 59.72 | |

Table.7 accuracy data for HPLC

Assay:

10 and 50 µg/ml of standard and samplesolutions were injected on UV and HPLC system using optimized mobile phase and other chromatographic condition. Chromatogram of standard solution (six replicate) and sample solution (two replicate) were recorded. Typical chromatogram of sparfloxacin was presented in fig. The concentration of sparfloxacin in the tablet formulation was calculated by comparing area of standard. The % assay of the drug was calculated and presented in table. 8

| Sample ID | Absorbance by UV | Area by HPLC | % assay (UV) | %assay (HPLC) |
|---|---|---|---|---|
| Standard | 0.573 | 2554108 | 100.6% | 99.30 |
| Sample | 0.577 | 2536214 | | |

Table.8 assay data

Marketed formulation

Zospar 200mg FDC Ltd

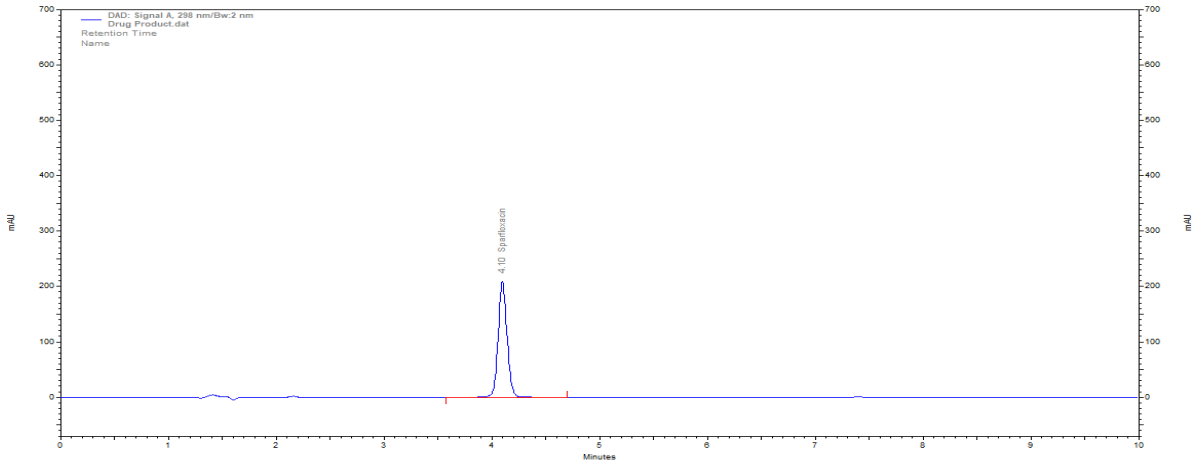


Fig.6 chromatogram of Zospar tablet (50µg/ml)

LOD & LOQ:

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

Robustness:

Robustness of the method was determined by taking slight changes in the chromatographic conditions, such as change in mobile phase, flow rate and column temp. It was observed that table.8 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 temp. Variation are well within the limit, which shows that the method having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2 %.

| Parameter by UV | Condition | RSD |
|---|---|---|
| Wavelength | 291nm | 1.72 |
| Lambda max= 293nm | 295nm | 1.01 |
| Parameter by HPLC | Condition | % assay |
| Flow rate (1ml/min) | 0.8mi/min | 99.55 |
| | 1.2ml/min | 99.06 |
| Mobile phase(75:25) | 77:23 | 99.32 |
| | 73:27 | 99.25 |

Table.9 robustness

## IV.DISCUSSION

In order to validate our proposed method, parameter such as detection wavelength, ideal mobile phase, optimum PH and concentration of standard solutions were exhaustively studied. The mobile phase consisting of methanol: water (60:40) and methanol: 0.1% TFA (25:75) was found to sharp, well defined peak with very good symmetry 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 1 ml/min was selected after preliminary test 4.10 min. the method was statistically validated as per ICH guideline. The analytical procedure was found to be linear in the concentration range 40-60 µg/ml with regression factor 0. 997.The percent RSD with respect to peak area, peak retention time and amount of sample solution less than 2 %. The recovery values obtained were between 99.70 % confirming accuracy of the proposed method. The LOD and LOQ were found to be and respectively. The method was satisfactory with respect to ruggedness and robustness.

## V.CONCLUSION

A simple, accurate, precise, method was developed for estimation of sparfloxacin in bulk and tablet dosage form. This method can be determining the purity of the drug available in various sources detecting the related degradation peak. The statistical analysis of data indicates that the method is reproducible, selective, accurate and robust.

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