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"METHOD DEVELOPMENT AND VALIDATION OF SPARFLOXACIN BY UV-VISIBLE SPECTROS-COPY."

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ABSTRACT:

The amount of sparfloxacin in bulk and pharmaceutical dosage formulations has been identified utilizing a straightforward, accurate, and cost-effective UV spectrophotometric Method using methanol: Water (60:40) as diluents. The drug was quantified at a specified λ max of 293 nm and proved linear in the range of 2 – 10 g/ml with good correlation coefficient (R^2=0.999) and great mean recovery (98- 100.9). LOQ and LOD were discovered to be 0.213 and 0.070 respectively. According to ICH guidelines, the method's linearity, accuracy, repeatability, and reproducibility were statically and by recovery experiments validated. The obtained results demonstrated that the Method can be used for the regular examination of sparfloxacin in bulk as well as in commercial formulations.

Keywords: Sparfloxacin, UV-Visible Spectroscopy, Method Validation, ICH.

INTRODUCTION

The advancement of analytical methods Analytical procedures are intended to determine the identification, purity, physical qualities, and potency of the medications in order to promote drug testing against specifications throughout manufacturing and quality release activities as well as during long term stability studies. [1, 2] An analytical method is validated when it has been proven through laboratory research that its performance characteristics are appropriate for the intended analytical uses. [3]

Sparfloxacin chemically: 5-amino-1-cyclopropyl-7-[(3R, 5S)-3, 5-dimethylpiperazine-1-yl]-6, 8-difluro4-oxoquinoline-3-carboxyic acid is fluoroquinolone antibiotics used for bacterial infections. Sparfloxacin works to kill germs by preventing DNA synthesis. A bacterium topoisomerase is called gyrase. Lower respiratory tract infections picked up in the neighborhood are recommended for treatment with the substance. Acute sinusitis, bacterially induced flare-ups of persistent bronchitis, and community-acquired Pneumonia. [4-6]

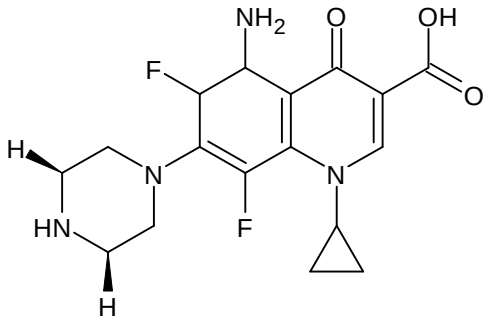

Figure1:- 5-amino-1-cyclopropyl-7-[(3R, 5S)-3, 5-dimethylpiperazine-1-yl]-6, 8-difluro4-oxoquinoline-3-carboxyic acid

Literature survey reveals thatA very few analytical methods found in literature survey for the determination of Sparfloxacin including FT-IR and UV-Spectrometry and also found few RP-HPLC methods [7-19]

In this study, an effort was made to create an affordable, quick, and straightforward UV spectrophotometric method for measuring the amount of sparfloxacin in raw materials and commercial dose formulations. The new method displayed good specificity, linearity, precision, and accuracy for Sparfloxacin and was optimised and validated in accordance with the International Council on Hormonisation (ICH) criteria.

MATERIAL AND METHOD

Instrument: A Shimadzu UV–visible spectrophotometer (UV1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells and electronic balance (Phoenix ISO 9001 company) was used.

Materials: All chemicals and reagents were of analytical grade. Sparfloxacin pure drug (API) was received by Rakshit pharmaceutical limited, Mumbai. Sparfloxacin tablets were purchased from local market.

Preparation of standard stock solution and working stock solution of sparfloxacin: 10 mg of sparfloxacin accurately weighed and dissolved in 10ml of methanol: water (60:40) in 50 ml conical flask. sonicate for for 10 min and transferred in 10 ml volumetric flask. 1000µg/ml solution was prepared. From standard stock solution 1 ml solution was pipette out and makes it up to 10 ml by methanol: water in 10 ml volumetric flask. 100µg/ml solution was prepared from this solution pipette out 0.2 ml, 0.4ml, 0.6ml, 0.8ml and 1 ml of solution and make upto 10 ml leads to 2µg/ml, 4µg/ml, 6µg/ml,8µg/ml, 10µg/ml concentration solution. This solution was estimated by UV spectrophotometer by using methanol: water (60:40) as blank at 293 nm.

Determination of wavelength of maximum absorption (λmax):

A standard stock solution of Sparfloxacin (100 µg/ml) was prepared using methanol: water (60:40) as solvent and 1 ml was diluted to 10 ml with the same solvent to obtain 10 µg/ml reference solutions. The reference solution was scanned in the wavelength region of 200-400 nm.

Method Validation:

Linearity and range

For linearity study from the working standard at different concentration 2, 4, 6,8,10µg/ml of drug solution were placed in 6 different 10 ml volumetric flask was made upto the mark with water. Absorbance was measured at 293nm and results are recorded in table.1 and fig.3 then obtained data used for the linearity calibration plot.

Accuracy and recovery study:

This study was carried out using the stock solution (100µg/ml).Results within the range of ensure an accurate method as well as indicate non- interference with the excipient of formulation. The accuracy of the given methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120% the recovery studies were carried out by adding known amount of sparfloxacin drug to reanalyzed tablet. The resulting solutions werethen reanalyzed by proposed methods; the results are reported in table.3

Precision: Prepare the standard stock solution of sparfloxacin. Prepare the three concentration of (2, 6, and 10µg/ml), by using mobile phase water. At the intraday and interday, take the maximum. Determine the % RSD. Analyzed were variations in findings within a day (intra-day) and variations in outcomes between days (interday). Sparfloxacin was examined three times at 293 nm for intraday precision on the same day. Inter-day precision was determined and analyzing the drug different day for three days at 293nm. Precision data for sparfloxacin at 293 nm is given table.4

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in the analytical procedure parameters. Change in wavelength ±2 nm. Change in flow rate ± 0.2 ml/min. robustness data reported in table.5.

System suitability:

System suitability is performed by UV-Spectroscopy and data is reported in table.6

RESULT AND DISCUSSION:

Method development and optimization: Sparfloxacin was made as a standard stock solution at a concentration of 100 g/ml in water as diluents. Pipette 1 ml into a volumetric flask that holds 10 ml to create a concentration of 10 g/ml from the stock solution mentioned above. The wavelength corresponding to the peak absorbance in the diluent of methanol: water (60:40) was determined to be 293 nm after the sample was produced and scanned in a UV Spectrophotometer from a range of 200-400 nm.

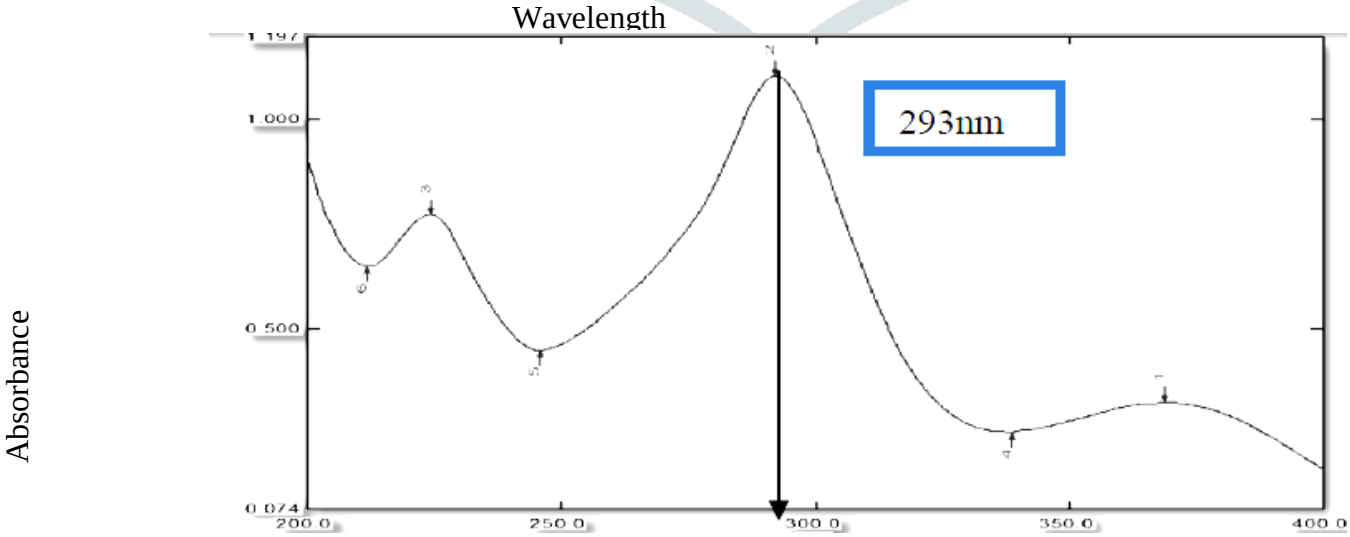

Figure 2.UV spectrum of Sparfloxacin

Linearity: Several aliquots of the drug's standard solution were made from stock solution and examined in order to demonstrate the linearity of the proposed approach. The medication demonstrated linearity with a correlation value in the 2–10 g/ml range. Table 1 displays linearity data.

Table1: Linearity data of sparfloxacin

Sr. No.	Conc.(µg/ml)	Absorbance
1	2	0.292
2	4	0.44
3	6	0.72
4	8	0.941
5	10	1.102

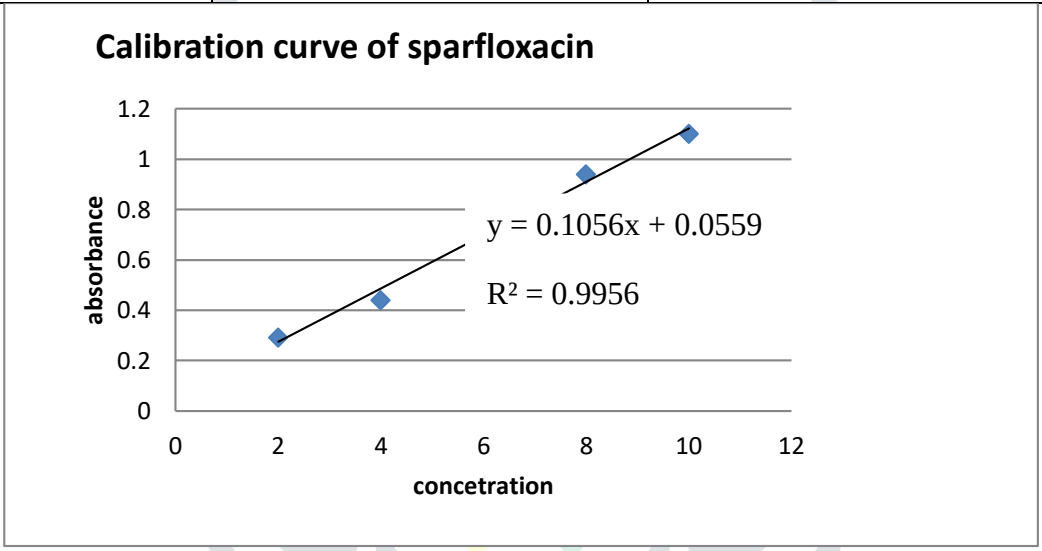

Figure 3 Calibration curve of sparfloxacin

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.070 µg/ml and0.2139 µg/ml respectively (Table 2) which indicates that the proposed UV method is sensitive.

Table 2: Result of LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Sparfloxacin	0.070	0.2139

Accuracy:

Recovery study results were within the range of 99.07-101.1 % indicating that the developed method is an accurate method for determination of niacin. The results are given in Table 3.

Table 3 : Accuracy data of sparfloxacin

Accuracy level	% Recovery	Statistical analysis	
		SD	%RSD
80%	99.07	0.145	0.8149
100%	98.20	0.305	1.5571
120%	101.1	0.109	0.4897

Precision: The developed method was found to be precised as the average % RSD values for intraday and inter-day precision study was found to be **0.92%**and **0.46%**respectively.

Table 4: Precision data of sparfloxacin

	Intra-day precision			Inter-day precision		
Conc.(µg/ml)	**2**	**6**	**10**	**2**	**6**	**10**
Absorbance	0.278	0.71	1.086	0.273	0.701	1.091
	0.277	0.707	1.088	0.278	0.708	1.092
	0.275	0.705	1.086	0.275	0.704	1.09
	0.280	0.711	1.09	0.276	0.705	1.091
	0.289	0.719	1.08	0.278	0.702	1.093
	0.286	0.701	1.088	0.279	0.709	1.094
Avg.	0.2808	0.71	1.0883	0.276	0.705	1.0918
SD	0.005	0.003	0.006	0.002	0.0031	0.004
%RSD	1.95	0.86	0.31	0.81	0.45	0.13

avg.% RSD	**0.92%**			**0.46%**		

Robustness: It was observed (Table: 5) that there were no significant changes in the results, which demonstrated that the developed method is robust**.**

Table 5: Robustness Data of Sparfloxacin

Change in wavelength	**(-2 nm) 291nm**			**(+2nm) 295nm**		
Conc.(µg/ml)	**2**	**6**	**10**	**2**	**6**	**10**
Absorbance	0.275	0.709	1.094	0.288	0.543	0.774
	0.275	0.709	1.094	0.282	0.545	0.776
	0.283	0.711	1.088	0.285	0.53	0.771
	0.288	0.718	1.091	0.289	0.548	0.769
	0.287	0.715	1.077	0.283	0.555	0.772
	0.280	0.709	1.093	0.283	0.532	0.768
Avg.	0.282	0.71	1.0883	0.285	0.54	0.771
SD	0.004	0.003	0.006	0.002	0.009	0.003
%RSD	1.72	0.49	0.56	1.01	1.76	0.39
avg.% RSD	**0.92%**			**1.053%**		

System Suitability: System suitability is performed by UV-Spectroscopy and data is reported in table.6

Table 6 : System Suitability of Sparfloxacin

Sr. No	**Absorbance**
1	1.094
2	1.09
3	1.088
4	1.091
5	1.077
6	1.093
Avg.	1.0883
SD	0.006
%RSD	0.56

Assay for Marketed formulation: In marketed formulation study we used zospar tablet for study of assay by using diluents methanol: water (60:40) and the following result obtained by using 10µg/ml concentration.

Table 7 Assay for Marketed formulation

Concentration	Absorbance
10	0.573
10	0.577
Mean	0.575
SD	0.0028
RSD	0.491
%Assay	100.6 %

CONCLUSION:

The model drug sparfloxacin's method development and validation were carried out utilising mobile water, which produces strong peaks and UV spectra as well. There are only a few analytical techniques, such as FTIR and UV spectrophotometry, that can be used to determine the presence of sparfloxacin. The developed UV and HPLC approach for simultaneous drug measurement in dosage forms was found to be straightforward, quick, accurate, and exact. The approach underwent a best-case linear regression analysis that looked at factors including linearity, robustness, accuracy, and precision. The % RSD for every parameter was discovered to be less than two, indicating the validity of the approach and the reasonable agreement of the assay results acquired by this method.

All results obtained with proposed method confirm the suitability of this method for the analysis of pharmaceutical dosage form. The developed method has been successfully applied for routine in process quality control.

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