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A new colorimetric determination and validation of Sparfloxacin based on ferric nitrate chromogen

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ABSTRACT

A new colorimetric method was developed for the estimation of Sparfloxacin in bulk and pharmaceutical dosage forms, based on the formation of a yellowish colored ferric ion complex with ferric nitrate solution. The yellow color may be due to the complex formation between the drug and added ferric ions. The absorption maximum for the above method was found to be 430nm. The formed chromogen obeyed beer's law in the concentration range of 20 – 100 µg/ml. The developed method was validated as per the ICH guidelines.

Key Words: Sparfloxacin, Chromophore, Absorbance.

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INTRODUCTION

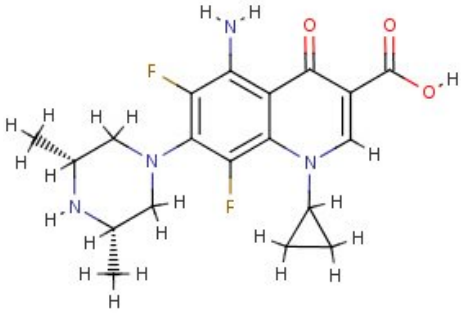

Fig.1.chemical structure of Sparfloxacin

Sparfloxacin is an Aminofloroquinolone with broad spectrum of antibacterial activity against gram positive and gram negative anaerobic bacteria with bactericidal effect. The chemical structure of Sparfloxacin was shown in fig.1. Chemically, Sparfloxacin is 5 – amino – 1 – cyclopropyl – 7 – (cis 3, 5 dimethyl – 1 – piperazinyl) -6, 8 difluoro 1, 4 dihydro – 4 –oxo – quinoline-3- carboxylic acid and it inhibits the DNA replication of bacteria by inhibiting DNA gyrase activity. Sparfloxacin is official in Martindale Extra Pharmacopoeia [1]. Literature survey reveals that, a variety of HPLC methods [2, 3], visible and UV Spectrophotometric methods [4-8] were developed for the determination of Sparfloxacin in different bulk and pharmaceutical dosage forms. Here in, an attempt made to develop a new sensitive and rapid spectrophotometric method for quantitative estimation of Sparfloxacin. The method was based on the formation of yellow colored chromogen with ferric ions of ferric nitrate solution. In the present communication, we were developed a simple visible spectroscopic method with considerable precision, accuracy and sensitivity for the estimation of Sparfloxacin in bulk and pharmaceutical dosage forms at 430 nm wavelength.

EXPERIMENTAL

Reagents & materials

The pure standard of Sparfloxacin was obtained as a gift sample from Aurabindo pharmaceuticals, Pvt.ltd, Hyderabad. The purity of the standard was found to be 99.86% and it was established by spectral confirmation. A shimadzu-uv-visible double beam spectrophotometer-1601, with matched cells was used for spectral measurements. All the chemicals which are used for performing of work were of A.R grade from S.D.FINE chem., Mumbai. The ferric nitrate solution and Sparfloxacin tablets was employed for this study. Ferric nitrate solution was prepared by dissolving the 1gm of ferric nitrate in 100ml of 3N nitric acid.

Preparation of working standard solution

Standard solution of Sparfloxacin was prepared by dissolving 100mg of Sparfloxacin in 100ml of distilled water to get 1mg/ml solution. The stock solution was made a series of dilutions (0.2-1.0) to 10 ml to get the concentrations of 20, 40, 60, 80, 100 µg/ml. These solutions were used to determine the parameters like, Absorption maximum (λ_{max}), Beer's law concentration and linearity.

Determination of λmax

20 µg/ml of Sparfloxacin bulk drug solution was scanned against reagent blank and the λmax was found to be 430 nm.

Beer's Law Concentration

ISSN: 0975-8585

Aliquots of standard solution ranging from 0.2-1.0 ml of Sparfloxacin were transferred into five separate 10 ml serially numbered volumetric flasks. To this 1ml of ferric nitrate solution and flasks were kept aside for 2 minutes for color development. Then appropriate volume of distilled water was added to each volumetric flask to bring the total volume to 10ml. The absorbance of the final yellow colored solution was measured at 430nm against reagent blank. A calibration graph was plotted and regression equation was calculated. The measured absorbances were plotted against concentration that reveals the beer's law Concentration lies between 20-100 µg/ml.

Preparation of sample stock solution: 10 tablets of each formulation F_1 and F_2 containing 200mg of Sparfloxacin were accurately weighed and powdered. A weight equivalent to 100mg of Sparfloxacin was weighed from the powdered tablets and transferred into a 100ml volumetric flask. 20ml of distilled water was added and shaken on a mechanical shaker for 15min. Then the volume is made up to 100ml with the same. It was then filtered through whatmann filter paper.

Assay of marketed formulation: From above stock solutions of each formulation of Sparfloxacin an aliquot of 0.2 ml was transferred into 10 ml volumetric flask. To this 1ml of ferric nitrate was added and kept aside for 2 minutes for color development. Then the volume was made up to 10ml with distilled water. The absorbance of yellow colored chromogen formed was measured at 430nm against the reagent blank. The amount of Sparfloxacin present in the sample solution was computed from the standard plot.

RESULTS AND DISCUSSIONS

The optimum conditions were established by changing one parameter at a time and keeping the others constant and by observing the effect produced on the absorbance of the colored species. Various parameters involved in the color development like, the concentration of the chromophoric reagents, volume and time involved for maximum color development were optimized. The linearity of method was optimized by standard plot and the method was obeying linearity in the range of 20-100 µg/ml. The yellow colored chromogen formed may be due to the complex formation in between the drug and added ferric ions. The optical characteristics such as beer's law limit, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope, Standard deviation, RSD and intercept of regression analysis were calculated for the proposed method and the results were incorporated in **table-1**.

ISSN: 0975-8585

Table. 1. Optical Characteristics and Regression Equation

S.NO	Parameter	Values	
		Sparfloxacin	Sparfloxacin (Tablet)
1.	λmax (nm)	430	430
2.	Beer's law (μg /ml)	20-100	20-100
3.	Regression equation	-	-
	a. Slope b. Intercept c. Correlation Co-efficient	0.0017 0.0003 0.999	0.0012 0.0007 0.996
4	Molar extinction coefficient (1 mole-1.cm-1)	0.067X 103	0.056 x103
5	Sandell's sensitivity (μg/cm2/0.001-absobance unit)	2.8846	2.761
6	% Range of errors** 95%Confidence interval 99%Confidence interval	± 0.00302 ± 0.0029	±0.00304 ±0.00225
7	% RSD	± 0.9177	±0.9323

***y= a + bc where c is the concentration of analyte and y is the absorbance unit**

Estimation of Sparfloxacin in marketed formulation

The assay for the marketed tablets of sparfloxacin was established with present optimized Spectrophotometric conditions and it was found to be more accurate and reliable. The results were shown in **table-2**.

Table. 2. Assay of Sparfloxacin

Drug	Label claim (mg/tab)	Amount estimated* (mg/tab)	Mean(±s.d) mean (mg) found by proposed method*	Coefficient of variance*
Sparfloxacin(F1)	200	199.8467	199.8467± 0.0350	0.6588
Sparfloxacin(F2)	200	199.9167	199.9167 ± .0308	0.5234

***Mean of five values**

Accuracy of the method

To study the accuracy, reproducibility of the proposed method, the recovery studies were carried out by addition of standard drug solution to preanalysed samples. Results of recovery studies were found to be satisfactory and were presented in **table- 3.**

ISSN: 0975-8585

Table. 3. Recovery Studies

Drug	Amount Added (mg)	Amount recovered* (mg)	% recovery
Sparfloxacin	20	19.87±0.46	99.35
	40	39.58±0.62	98.95
	60	59.96±0.35	99.93

***Mean of five values**

Precision of the method

The intraday and inter-day variations of the method were determined using six injections of three concentrations and they are analysed on the same day and three different days over a period of two weeks. The result s obtained was satisfactory and they are lying within the limits. Results were shown in **table-4**.

Table. 4. Precision

Drug name	Concentration (µg/ml)	Observed concentration*			
		Intraday	%RSD	Inter day	%RSD
Sparfloxacin	20	20.02	0.34	19.92	0.42
	40	39.96	0.12	40.13	0.67
	60	60.04	0.56	60.19	0.82

***Mean of six values**

Conclusion

The results indicate that the above proposed methods were simple, rapid and sensitive with reasonable precision and accuracy which makes it as choice for routine quality control analysis. There was no interference of excipients present in tablet formulation through out the experimental process that reflects the accuracy and precision of method.

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