Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(1): 528-532

ISSN No: 0975-7384

Development and Validation of Spectrophotometric Methods for the Estimation of Sparfloxacin in Tablet Dosage Forms

Patel Kamlesh*, Badmanaban R, Patel CN

Shri Sarvajanik Pharmacy College, Near Arvind Baug, Mehsana-384001, Gujarat, India

Abstract

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of sparfloxacin, in bulk drug and pharmaceutical dosage forms. Methods were based on the formation of coloured chromogens, which were measured at 549 nm and 465 nm, respectively. The results obtained with the proposed methods are in satisfactory with the labeled amounts when tablet dosage forms were analysed.

Key-words: Sparfloxacin, Visible spectroscopy, Diazotization, ß-naphthol, P-dimethylamino cinnamaldehyde (PDAC).

Introduction

Sparfloxacin is chemically, 5-amino-1-cycloproyl-7-[(3R, 5S) 3, 5- Dimethylpiperazine-1-yl]-6,8-difluro-4-oxo-quinoline-3-carboxylic acid. It is an antibacterial and anti tubercular drug. The anti bacterial action of Sparfloxacin results from inhibition of the enzyme topoisomerase II (DNA gyrase) and topoisomerase IV which are required for bacterial DNA replication, transcription repair and recombination. Sparfloxacin is not official in any pharmacopoeia.

Materials and Methods

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of sparfloxacin by using ß-naphthol and PDAC at room temperature, respectively.
Method A is based on the diazotization of Sparfloxacin with nitrous acid to form diazotized sparfloxacin, with ß-naphthol to form a red colored chromogen which shows maximum absorption at 549.0 nm and obeys beer's law in the concentration range of 5-25µg/ml.

Method B is based on condensation of Sparfloxacin with P-dimethylamino cinnamaldehyde to form Schiff's base that is yellow colored chromogen which shows maximum absorbance at 465.0nm and obeys beer's law in the concentration range of 10-50µg/ml.

A Shimadzu UV/Visible double beam spectrophotometer (model 1700) with 1 cm matched quartz cells was used for all spectral measurements. Scanning range of 190-380nm for UV range and 380-800nm for visible range were used. All chemicals used were of A.R. grade from S.D. Fine-Chem. Mumbai. Sparfloxacin was kindly gifted by Dr.Reddy Labs Pvt. Ltd, Hyderabad.

Pure Sparfloxacin powder equivalent to 100 mg was accurately weighed and dissolved in 50 ml of 0.1N NaOH in a 100 ml volumetric flask and the volume was made up to 100 ml with distilled water (1 mg/ ml).From this a working standard solution containing 100µg/mg was prepared with distilled water for both the methods. Twenty tablets of two different brands of Sparfloxacin were weighed and powdered in glass mortar and the powder equivalent to 25 mg of Sparfloxacin was weighed accurately and transferred into a 25 ml standard volumetric flask. The contents were dissolved in 0.1N NaOH and sonicated for five minutes. This solution was filtered through 0.45 µWhatmann filter paper. 5 ml of the filtrate was diluted to 50 ml with 0.1N NaOH to get the solution of 100µg/ml. An aliquot of 1 ml of test solution was diluted to 10 ml with 0.1N NaOH so that to produce the concentration 10µg/ml.

Procedure
In method A, Aliquots of standard solution of Sparfloxacin ranging from 5 to 25µg/ml were transferred into series of 10 ml volumetric flasks .To each flask 1.0 ml of hydrochloric acid (2N)and 1.0ml of sodium nitrite (0 .1%w/v) added and a reaction time of 10 min at 0-5º C was given for the completion of reaction.

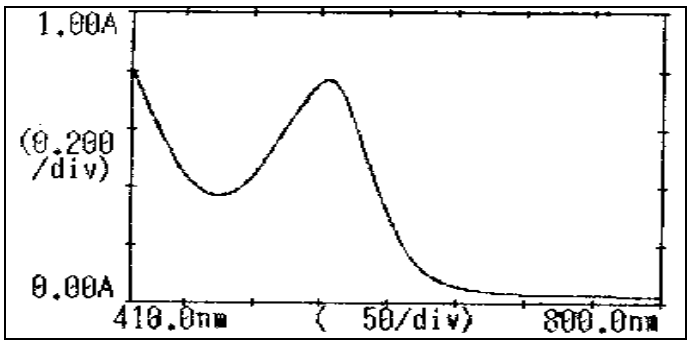

Figure 1 Absorption Spectrum of Sparfloxacin with β-naphthol

Then 1.0ml of alkaline β -naphthol solution (0.1%w/vin 2%aqueous NaOH) was added to each flask with gently shaking and after10 min , the volume in each flask made up to 10 ml with distilled water . The absorbance of red colored chromogen were measured at 549.0 nm against the reagent blank .The colored chromogen was stable for 1hr. The amount of Sparfloxacin present in the sample solution was computed from the respective calibration curve.

In method B, Aliquots of standard solution of Sparfloxacin ranging from 5 to 25µg/ml were transferred in to a series of 10 ml volumetric flasks .To each flask, 0.5 ml of ferric chloride solution (0.3%w/v) and 0.1 ml of 1 10-phenathrolione (0.3%w/v) was added and heated on boiling water bath for 15 min at a temperature of 70ºC the flasks were cooled and the volume in each flask was made up of 10 ml with distilled water. The absorbance of red colored chromogen were measured at 465.0 nm against the reagent blank .The absorbance of red colored chromogen was stable for 1hr. The amount of Sparfloxacin present in the sample solution was computed from the respective curve.

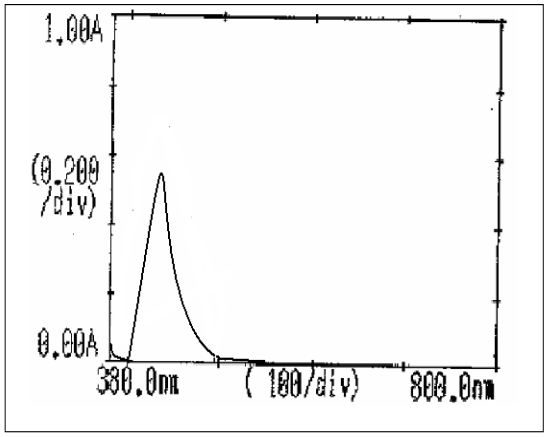

Figure 2 Absorption Spectrum of Sparfloxacin with PDAC

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1. The regression analysis using method of least squares was performed for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table 1. The percentage relative standard deviation and percentage of error (0.05 and 0.01 level of confidence limit) calculated from Mean of eight determinations. Beer's law limits of sparfloxacin are given in Table 1. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for the dosage forms Table 2 confirms the suitability of the methods for pharmaceutical dosage forms when compared to UV method. The proposed methods are reaction specific and eliminate interference from impurities.

The optimum conditions for colors development for methods A and B were established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of the coloured species and incorporated in the procedures.
To evaluate the validity and reproducibility of the methods, known amounts of the pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed methods. The percent recoveries are given in Table 2. Interference

studies revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium trisilicate, that are usually present in the tablet dosage forms did not interfere at their regularly added levels.

Results

Two new, sensitive and most economical analytical colorimetric methods were developed for estimation of Sparfloxacin in bulk and pharmaceutical dosage forms.

Table 1 Optical characteristics and other Parameters of Method

Parameter	ß-naphthol	PDAC
λ_{max} (nm)	549	465
Beer's law limits (µg/ml)(x)	5-25	10-50
$E_{cm}^{1\%}$	137	95
Regression equation (Y*)	Y=0.0391X– 0.0024	Y=0.0193X0.0038
Slope (m)	0.0391	0.0193
Intercept (c)	0.0024	0.0038
Correlation coefficient(r^2)	0.9974	0.9962
LOD (µg/ml)	1.15	2.7
LOQ (µg/ml)	3.45	8.1

*Y = mx +c were x is the concentration of Sparfloxacin in µg/ml and Y is the absorbance at the respective λ_{max}

Table 2 Validation Parameters

PARAMETER	VALUES	
	ß-naphthol	PDAC
Amount found ± %RSD*	199.69 ± 0.81	199.46 ± 0.72
%Content ± %RSD*	99.843 ± 0.81	99.73 ± 0.72
Intra Day Amount Present ± %RSD*	100.01 ± 0.98	99.13 ± 1.33
Inter Day Amount Present ± %RSD*	98.13 ± 1.10	98.67 ± 0.98
% Recovery ± %RSD*	99.87 ± 0.23	99.73 ± 0.19
Robustness ± %RSD*	97.10 ± 0.21	99.10 ± 0.23
Ruggedness ± %RSD*	98.11 ± 0.78	98.29 ± 0.31

* n= 5 replicate determinations

These methods are validated in terms of sensitivity, accuracy and precision. The results were found to be accurate, and free from interference from tablet excipients. The active pharmaceutical ingredient was extracted from its finished dosage form using hot water and HCl. The % recovery, linearity, LOD and LOQ, Sandell's sensitivity and molar absorptivity for sparfloxacin are summarized in Table 1 & 2.

Conclusion

The UV spectroscopic methods and colorimetric methods demonstrated herein, are applicable to the estimation of Sparfloxacin in pure as well as in existing dosage forms. In order to ensure that the data generated each of the above methods are both accurate and precise. The experiments have been performed on calibrated equipments using suitable reference standards. To prove and documents the reliability of the methods have been carried out to a possible extent. The results expressed in Table 1 & 2 for spectrophotometric methods. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical.

The proposed methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of sparfloxacin in bulk drug and its pharmaceutical dosage forms (tablets) in a routine manner.

Acknowledgements

The authors thank the S.D. fine- chem., Mumbai. Sparfloxacin was kindly gifted by Dr.Reddy Labs Pvt. Ltd, Hyderabad for research and Shri Sarvajanik Pharmacy College, Mehsana management, for providing all facilities to carry out the present work.

References

[1] BK Sharma. Instrumental methods of chemical analysis, In; Introduction to Analytical chemistry, 23rd Edition, Goel Publishing House, Meerut, **2004**; 1-4.
[2] Willard HH, Merritt LL, Dean JJA, Frank AS. Instrumental method of analysis. 7th Edition, CBS Publishers and Distributors, New Delhi**, 1986**; 321-23.
[3] Higuchi T, Brochman–Hausen E. Pharmaceutical Analysis. 6th Edition, Interscience, London**, 1961**; 433-40.
[4] Siggia S, Hanna JG. Quantitative organic Analysis via Functional Groups. 4th Edition, Interscience, Nottingholm, **1979;** 231-35.
[5] Michael E, Schartz IS, Krull. Analytical method development and Validation. **2004**; 25-46.
[6] Berry RI, Nash AR. Pharmaceutical Process Validation; Analytical method validation. Marcel Dekker Inc, New work, **1993,** 57, 411-28.
[7] Elhance DN. Foundation of statistics. Kitab mahal, Cheronics ND, Ma TS. Organic Functional Group Analysis by Micro and Semi micro Methods. 47th Edition, Interscience, New York, **1964**, 20, 229.
[8] Hunig S, Balli H, Briether E, Bruhne F, Geiger H, Grigat E, Muller F, Quast H. *Angew Chem.* **1962**, 74, 818-24.
[9] Sawicki E, Hauser TR., Stanley TW, Elbert W. *Anal Chem.* **1961**, 33, 93-6.