

Development of UV-Spectrophotometric Method for Assessment of Sparfloxacin in Bulk and Pharmaceutical Dosage Forms

Kaushal Kumar*, Lakshyaveer Singh

Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, India

ABSTRACT

The aim of this study was to develop and validation of an UV-spectrophotometric method for the estimation of sparfloxacin in bulk and various pharmaceutical dosage forms using 0.1 N HCl (gastric simulatory media) as per ICH guidelines. The λ_{max} for sparfloxacin in gastric simulatory media was found to be 296 nm. The drug followed linearity at the concentration range of 2-12 $\mu\text{g/ml}$ with a correlation coefficient value of 0.999. The devised method was applied onto the bulk and tablets (marketed pharmaceutical formulation) for quantification of contents of drug. Estimated drug was found to be in-between 98.43 to 101.57 % in marketed tablets, a good agreement with the claimed level. The accuracy of the method was verified by recovery experiment performed at three different levels- 75%, 100%, and 125%. The % recovery was found to be in the range of 97.31-101.45%. The accuracy and reproducibility of the method was indicated by low values of % RSD. The precision of the method was studied through intraday and Interday variations and repeatability. The % RSD value < 2 confirmed the precision of developed method. Ruggedness of the proposed method was studied with the help of two analysts. The proposed method was found to be a rapid yet successful tool for routine analysis of sparfloxacin in the bulk and in the pharmaceutical dosage forms.

Keywords : Fluorquinolone, sparfloxacin, Quantitative determination, UV, validation

I. INTRODUCTION

Sparfloxacin^[1,2], 1,4-dihydro-5-amino-1-cyclopropyl-6,8-difluoro-7-(3,5-dimethyl-1-piperazinyl)-4-oxo-,cis-3 Quinolinecarboxylic acid [Figure-1], is a potent synthetic antibiotic of fluoroquinolone class which has in vitro activity against a wide range of Gram-negative and Gram-positive microorganisms^[3,4,5]. Sparfloxacin exerts its antibacterial activity by inhibiting DNA gyrase, a bacterial topoisomerase^[6].

Only few analytical methods have been reported for determination of Sparfloxacin in bulk and pharmaceutical formulations. Such methods include colorimetry ^[7,8], HPLC ^[9], RP-HPLC ^[10] and UV-densitometric^[11]. However, UV-spectrophotometric estimation of sparfloxacin in **biological fluids** hasn't been reported yet. Because of its simplicity, economy, specificity and easy availability

spectrophotometry continues to be a popular method of drug estimation among researchers. The objective of this study was to develop and validate an UV-spectrophotometric method for the estimation of sparfloxacin in 0.1 N HCl (gastric simulatory media) for bulk and pharmaceutical formulations as per ICH guidelines ^[12]. Present study has come up with a reliable spectrophotometric method for determination of sparfloxacin in gastric simulatory media in bulk and pharmaceutical formulations.

II. EXPERIMENTATION

Instruments

The present work was carried out on 1601 series model UV-Visible Double Beam Spectrophotometer of Shimadzu, Japan. The absorption spectra of reference and test solutions were carried out in a 1 cm quartz cuvette.

III. MATERIALS AND METHODS

Materials

Sparfloxacin crude drug sample (A.P.I.) was supplied by wockhardt limited, Bandra-Kurla complex, Mumbai.

Tablets (Spardac-DT 200mg by zydus cadila) were purchased from the local medical store.

All other chemicals and reagents used were of analytical grade and purchased from Central Drug House, New Delhi, India.

Preparation of standard stock solution

Accurately weighed 20 mg of sparfloxacin was dissolved in 0.1 N HCl by shaking manually for 10 minutes and the volume was made upto 100 ml. This solution (200µg/ml) was then diluted 10 times to give the stock solution of a concentration equal to 20µg/ml.

Selection of wavelength for analysis of sparfloxacin

For finding wavelength of maximum absorbance (λ_{max}) for sparfloxacin, an U.V. scanning of appropriate volume of 10µg/ml solution of sparfloxacin in 0.1 N HCl was carried out in spectrophotometer between the scanning range of 200 to 400nm. The λ_{max} for sparfloxacin in 0.1 N HCl was found to be 296nm.

Preparation of calibration curve of sparfloxacin in 0.1 N HCl

From stock solution different aliquots in the range of 1–6 ml were taken and diluted up to 10 ml with 0.1 N HCl to get concentrations 2.0, 4.0, 6.0, 8.0, 10 and 12 µg/ml respectively. UV absorbances were taken for the diluted samples at wavelength of maximum absorbance (λ_{max})- 296nm using 0.1 N HCl as blank. Each experiment was repeated three times and averages were calculated and calibration curve was plotted between concentrations (µg/ml) and absorbances.

METHOD VALIDATION

Validation of the developed method was done as per ICH guidelines in terms of parameters like linearity and range, accuracy, precision, specificity, ruggedness, robustness etc [13, 14].

Linearity and range For finding out the concentration range over which the drug solutions show absorbance as a linear function of concentration, different aliquots of sparfloxacin stock solution were diluted with 0.1 N HCl to get various concentrations from 1 to 20 µg/ml. Absorbances of the solutions were observed on UV spectrophotometer at λ_{max} 296 nm. Absorbances with negative value and values more than 1 were excluded. Absorbance for each concentration was observed three times and averages were calculated. Calibration curves for different ranges of concentrations- 1 to 10 µg/ml, 2 to 12µg/ml, 2 to 20µg/ml were plotted between concentrations (µg/ml) and absorbances. Regression equations and regression coefficients were computed to study linearity.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. To evaluate the accuracy of the proposed method, recovery tests were carried out. Recovery tests were performed by adding known amounts of standard solutions (75%, 100% and 125%) to the pre-analysed sample and then reanalyzed by using the proposed method. The recovery studies were performed three times for each addition and percentage recovery and standard deviation of the percentage recovery were calculated.

Precision

Precision of proposed method was validated through estimation of intraday and interday variations. Intraday precision was determined by analyzing the 4, 6 and 8 µg/ml of sparfloxacin solutions for six times in the same day. Interday precision was determined by analyzing 4, 6 and 8 µg/ml of sparfloxacin solutions daily for 3 days. Average concentrations and %RSD were calculated.

Repeatability

Repeatability was determined by analyzing 8 µg/ml concentration of sparfloxacin solution for six times.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots of 4 µg/ml concentration by two analysts under similar environmental and operational conditions.

Estimation of sparfloxacin in bulk

Accurately weighed 10 mg of sparfloxacin was transferred into a 100 ml volumetric flask and the volume was made up to the mark with 0.1 N HCl. 1.0 ml of this solution was diluted with 0.1 N HCl to 10 ml. Absorbance for the resulting solution was observed at 296nm and its concentration was calculated from linear regression equations.

Estimation of Sparfloxacin in dosage form (Tablet)

20 tablets, each containing 200mg of Sparfloxacin, were weighed and their mean weight was calculated. These 20 tablets were finely powdered and powder equivalent to 10mg of sparfloxacin was transferred into a 100ml volumetric flask and dissolved in 0.1 N HCl. Undissolved excipients were filtered out and volume was made up to the mark with 0.1 N HCl. 1.0 ml of this solution was diluted with 0.1 N HCl to 10ml. Absorbance for the resulting solution was observed at 296nm and its concentration was calculated from linear regression equations. Experiment was performed in triplicate and the average was calculated.

IV. RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines by performing procedures given in the experimentation.

Linearity studies

Linear regression equation was found to be $y = 0.078x - 0.016$. Regression coefficient value 0.999 for concentration range between 2 to 12 $\mu\text{g/ml}$ confirmed the linearity in this range for sparfloxacin in 0.1 N HCl.

The results have been shown in Table 1. The linearity curve of sparfloxacin is presented in Figure 2.

Accuracy

Under recovery study percentage recoveries and their standard deviation from the reanalysis of spiked drug solution have been presented in Table 2. The lower values of % RSD (< 2) for recovery studies indicate the accuracy of the proposed method.

Precision

Through intraday precision and interday precision studies average concentrations and %RSD were calculated. % RSD was found to be less than 2%. The results have been shown in Table 3.

Repeatability

Low Value of %RSD (less than 2) validated the repeatability of the proposed method. The results have been shown in Table 4.

Ruggedness

The results have been shown in Table 5. Again Low Value of %RSD (less than 2) validated the ruggedness of the proposed method.

Estimation of sparfloxacin in bulk

The results have been shown in Table 6. The % RSD ($=0.92$) was less than 2%. [Table 6].

Estimation of Sparfloxacin in dosage form

The results have been shown in Table 7. The % of labeled amount in tablets was found in between 98.43 and 101.57 %. No interference from excipients was found [13, 14].

V. CONCLUSION

Thus the proposed method for estimation of Sparfloxacin for bulk as well as pharmaceutical formulation (tablets) in gastric simulatory media (0.1N HCl) was found to be simple, accurate, economical and rapid. The % RSD values of all parameters were found to be less than 2% in the developed method. The validation of the proposed method confirms it as an appropriate method for the quantification and routine analysis of sparfloxacin in bulk. It can also be used in routine quality control of the formulations containing sparfloxacin.

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VII. REFERENCES

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Figures and Tables

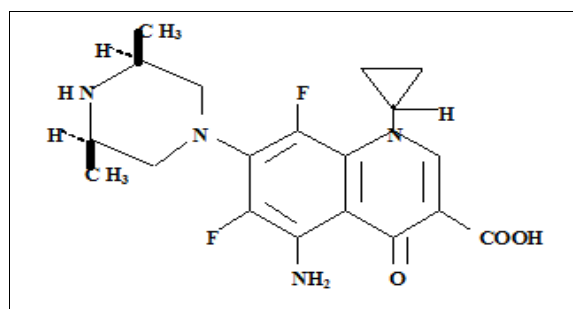


Figure 1: Chemical structure of sparfloxacin

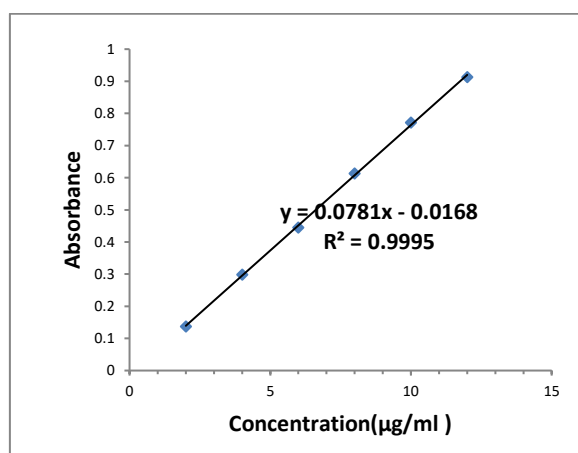


Figure 2: Calibration curve of sparfloxacin in 0.1 N HCl

Table 1: Linearity Parameters

Parameters	Values
Linearity range (µg/ml)	2-12 µg /ml
Regression coefficient	0.999
Slope	0.078
Intercept	-0.016

Table 2: Recovery studies

Test Concentration (µg/ml)	Amount Of Concentration Spiked		Avg. amount of sample from calibration graph	Amount Of Sample Recovered (µg/ml)	% Recovery	% RSD
	%	(µg/ml)				
4	75	3	7.028	3.028	100.94	0.94
		3	6.988	2.988	99.62	
		3	7.043	3.043	101.45	
4	100	4	8.003	4.003	100.06	1.39
		4	7.892	3.892	97.31	
		4	7.951	3.951	98.78	
4	125	5	8.976	4.976	99.51	0.50
		5	9.010	5.010	100.21	
		5	9.024	5.024	100.49	

Table 3: Precision studies

S. No	Concentration (µg/ml)	Intraday precision (n=6)		Interday precision (n=3)	
		Average Conc. found	%RSD	Average Conc. found	%RSD
1.	4	4.03	1.66	4.09	1.57
2.	6	6.05	1.30	6.03	1.49
3.	8	7.99	1.44	7.95	1.38

Table 4: Repeatability studies

S. No	Concentration (µg/ml)	Conc. found	%RSD
1.	8	8.05	0.97
2.	8	7.96	
3.	8	7.83	
4.	8	7.99	
5.	8	7.88	
6.	8	7.95	

Table 5: Ruggedness studies

Ana lyst	S. No	Conc. (µg/ml)	Conc. found	Average conc.	% RSD
KK*	1.	4	4.03	4.01	1.6
	2.	4	3.99		
	3.	4	4.01		
LVS*	1.	4	4.00	3.95	
	2.	4	3.89		
	3.	4	3.95		

KK*=Kaushal Kumar; LVS*=Lakshyaveer Singh

Table 6: Estimation of sparfloxacin in bulk

S. No	Concentration (µg/ml)	Conc. found	%RSD
1.	10	10.05	0.94
2.	10	10.13	
3.	10	9.89	
4.	10	10.02	
5.	10	10.11	
6.	10	9.94	

Table 7: Estimation of sparfloxacin in tablets

S. No	Amount of Drug (mg/tab)		% of claimed	%RSD
	Labelled	Estimated		
1.	200	196.86	98.43	1.67
2.	200	198.06	99.03	
3.	200	203.13	101.57	