Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2018, Volume 6, Issue 04
ISSN: 2321–3647(online)

Development and Validation of UV-Spectrophotometric Method for Estimation of Sparfloxacin In Gastric Simulatory Media In Bulk and Pharmaceutical Dosage Forms

Kaushal kumar
Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly.

ABSTRACT

The main 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) as per ICH guidelines. The λmax for sparfloxacin in 0.1 N HCl was found to be 296 nm. The drug follows linearity in-between the concentration range of 2-12µg/ml with a correlation coefficient value of 0.999. The proposed method was applied onto the bulk and marketed pharmaceutical formulation (tablets) so as to find out 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.

*Corresponding Author Email: saxena_kaushal@yahoo.co.in
Received 24 March 2018, Accepted 04 April 2018

Please cite this article as: Kumar K., Development and Validation of UV-Spectrophotometric Method for Estimation of Sparfloxacin In Gastric Simulatory Media In Bulk and Pharmaceutical Dosage Forms. American Journal of Pharmacy & Health Research 2018.

INTRODUCTION

Sparfloxacin[1,2],1,4-dihydro-5-amino-1-cyclopropyl-6,8-difluoro-7-(3,5-dimethyl-1- iperazinyl)-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 grace, a bacterial topoisomerase[6].

Only few analytical methods have been reported for determination of Sparfloxacin in bulk and pharmaceutical formulations. Such methods include colorimetric [7, 8], HPLC [9], RP-HPLC [10] and UV-densitometry[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.

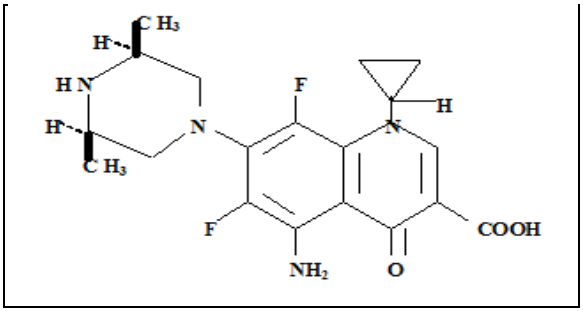

Figure 1: Chemical structure of sparfloxacin

MATERIALS AND METHOD

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.

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 up to 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 (λmax) 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 λmax 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 (λmax)-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. Absorbance of the solutions were observed on UV spectrophotometer at λ_{max} 296 nm. Absorbance 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-analyzed 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. Interday precision was determined by analyzing the 4, 6 and 8 µg/ml of sparfloxacin solutions for six times in the same day. Intraday 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 equation. Experiment was performed in triplicate and the average was calculated.

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 µg/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.

Table 1: Linearity Parameters

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

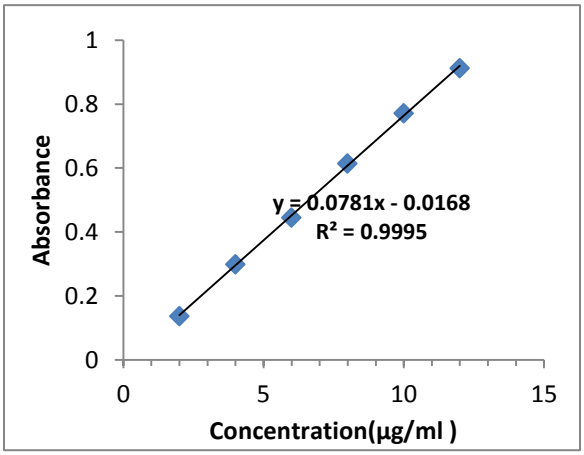

Figure 2: Calibration curve of sparfloxacin in 0.1 N HCl

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.

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	

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.

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

Repeatability

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

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	

Ruggedness

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

Table 5: Ruggedness studies

Analyst	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

Estimation of sparfloxacin in bulk

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

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	

Estimation of Sparfloxacin in dosage form

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

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	

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.

ACKNOWLEDGMENTS

The author is thankful to his colleague Lakshyaveer Singh for his kind co-operation and help in performing ruggedness studies as another analyst. Author is also thankful to Head of the department as well as other staff members for providing the required facilities and help needed to carry out this research work.

REFERENCES

1. www.rxlist.com
2. The Merck Index. 12th ed. New Jersey, USA: 8879
3. Canton E, Peman J, Jimenez MT, Ramon MS, Gobernado M. In vitro activity of sparfloxacin compared with those of five other quinolones. Antimicrob Agent chemother. 1992; 36(3): 558-565.

4. Kimura M, Kishimoto T, Hashiguchi K, Niki Y, Soejima R. In-vitro and In-vivo activities of sparfloxacin against Chlamydial species. Drugs. 1993; 45(S3):370.
5. Kocagoz SS, Gur D, Uzun O, Ozkuyumeu C,Akova M, Akalin HE. Comparative In-vitro activity of sparfloxacin against gram-positive cocci. Drugs. 1993;45(S3):199-200.
6. Andersson MI, MacGowan AP. Development of the quinolones. J Antimicob Chemother. 2003; 51(S1): 1-11.
7. Marona HRN, Schapoval EES. Spectrophotometric determination of sparfloxacin in pharmaceutical formulations using bromothymol blue. J Pharma biomen Anal. 2001; 26: 501-504.
8. Sujitha Parimala S, Asha Jyothi P, Tejaswi K. Spectrophotometric Estimation of Sparfloxacin in Bulk and Tablet Dosage Form. International Journal of Innovative Pharmaceutical Research. 2013,4(2),306-309.
9. Rahman NN, Ahmad S. Development of quantitative analysis of sparfloxacin by high performance liquid chromatography'. Dhaka University Journal of Pharmaceutical Sciences, August 2007:6(1).
10. Bera A K, De A K, Pal B. Development and validation of a rapid RP-HPLC method for estimation of sparfloxacinin tablet dosage form. International Journal of Pharmaceutical Sciences and Research. 2014; Vol. 5(2): 563-567.
11. Sharma M C, Sharma S. UV-Densitometric determination of sparfloxacin and its application to the assay in pharmaceutical dosage forms. Journal of Optoelectronics and Biomedical Materials Vol. 2, Issue 4, October-December 2010: 191 – 195.
12. ICH-Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005.
13. United States Pharmacopeia. USP Convention, Rockville, MD, 2008; p 1225.
14. ICH (Q2, R1).Note for guidance on validation of analytical methods: Definitions and terminology. International conference on harmonization.1994:9-13.