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Method Development and Validation of UV-Spectrophotometric Method for Quantitative Estimation of Ofloxacin in Pharmaceutical Dosage Form

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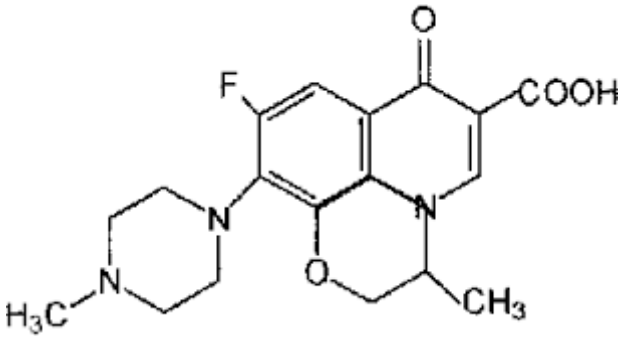
ABSTRACT

The aim of present work is to develop and validate simple, sensitive, economical and accurate Spectrophotometric method has been developed for determination of Ofloxacin in pure form and in pharmaceutical formulations. Ofloxacin in methanol shows maximum absorbance at 294 nm. The drug obeyed Beer's law in the concentration range of 15µg/ml in methanol. The proposed methods were successfully applied for the determination of drug in commercial tablet preparations. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Analysis, Method validation, Ofloxacin, Ultraviolet Spectroscopy.

INTRODUCTION

Ofloxacin is a quinolone / fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required replicating one DNA double helix into two[1]. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian [2]. Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [3].



Structure of Ofloxacin

Ofloxacin is soluble in aqueous solutions with pH between 2 and 5. Sparingly to slightly soluble in aqueous solutions with pH 7 (solubility falls to 4 mg/mL) and freely soluble in aqueous solutions with pH above 9. Ofloxacin acts on DNA gyrase and toposiomerase IV, enzymes which, like human topoisomerase, prevents the

excessive supercoiling of DNA during replication or transcription. By inhibiting their function, the drug thereby inhibits normal cell division [4].

Literature survey reveals that several analytical methods have been reported for the estimation of Ofloxacin by HPLC method. Apart from above no other work in the literature reported about the UV Spectrophotometric method for the analysis of Ofloxacin in pharmaceutical formulations. Thus there is need to develop simple and economical method for routine analysis of Ofloxacin. The objective of present study was to develop and validate simple, accurate, precise, robust and economical method for estimation of Ofloxacin in bulk and pharmaceutical formulations as per ICH Guidelines [5].

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade and HPLC grade procured from Qualigens, India Ltd. The chemicals used for the study Methanol (HPLC grade) Acetonitrile (HPLC grade) Water (HPLC grade) Methanol (Analytical grade) and DMSO (Analytical grade)

Methods

System precision/System Suitability

System suitability testing is an integral part of many analytical procedures. System suitability test parameters depend on the type of procedure being validated. System precision is determined by measuring the absorbance of standard solution containing 100% working concentration for six times and calculates the % RSD. The % RSD should be less than 2.0%. The relative standard deviation of six replicate measurement of standard solution was found to be 0.740% (limit NMT 2.0%), which indicates that the system is precise to analyze the sample [6].

Method Precision

Method precision was established by analyzing six separate samples at 100% of the working concentration. Percent of result was calculated against claimed label [7]. The % RSD of assay result of six separate samples from a single batch was found to be 0.291% (limit NMT 2.0%) which indicates that the method is precise to analyze the tablet

(Table-2).

Accuracy

Accuracy was established by analyzing nine sample solutions of Ofloxacin at 80%, 100% and 120% of the working concentration (Three replicates for each concentration) into a placebo mixture and by calculating the percent recovery of active ingredient from the placebo solution. The percent recovery at each level should be within 97.0% to 103.0%. A linear curve was prepared by plotting amount added Vs amount recovered correlation co-efficient. The percent recovery was calculated for nine determinations and found to be within limit. A graphical representation between amount added Vs amount recovered also shows linearity[8]. Thus it has been concluded that the method is accurate to analyze the Tablet **(Table-2)**.

Specificity Identification

The UV absorption spectrum of the sample preparation for assay is concordant with the reference spectrum of standard sample from assay preparation [9].

Placebo Interference

Placebo solution was prepared in the same manner as standard and sample preparation. No interference of placebo was found [10].

Linearity

Five different standard solutions were prepared covering a concentration of 80% to 120% of the working concentration of Ofloxacin and all absorbance were recorded. A linear curve was prepared by plotting actual concentration (µg/ml or ppm) Vs absorbance and correlation co-efficient was calculated. The results obtained correlate with the concentrations resulting in the following calibration curve. The correlation co-efficient found 0.9915, which is within the limit (limit: NLT 0.990) [11]. Thus the graph confirms the linearity of the method in the range of 80% to 120% **(Table-4)**.

Robustness:

Robustness of this method was determined by analyzing the Ofloxacin Tablet in different equipment in different day and by different analyst [12]. From the above-mentioned data it observed that the method is robust enough to analyze Ofloxacin Tablet **(Table-5).**

Recovery studies

In order to ensure the accuracy of the proposed method, recovery studies were carried out. To 50 % of the pre-analyzed sample solution, a definite concentration of 6.4, 8 and 9.6 µg/ mL standard solution of Ofloxacin and 3.2, 4 and 4.8 µg/ mL The absorbance of resulting solutions was measured at their corresponding wavelengths and the percentage recovery was calculated[13].

Limit of Detection and Limit of Quantification

The linearity studies were carried out for six times. The limit of detection and limit of quantification were calculated by using the average of slope and standard [14].

RESULT AND DISCUSSION

The analytical method development and validation for the drug Ofloxacin was done, which shows the best elution of the peak. The specificity test studies shows that the analyte chromatographic peak is not attributable to more than one component. The linearity calibration curve shows linear response over the range of concentration used. The precision data shows that the reproducibility of the assay procedure was satisfactory. The accuracy of the method was determined by recovery studies. The recovery studies were carried out of the percentage recovery was calculated. The Robustness studies show that there were no marked changes in the chromatogram. The Ruggedness of the method was determined for the same sample under different laboratory, different analysis and using operational and environmental conditions; the degree of reproducibility will shows results within their limits. Further there was no interference due to excipients. The system suitability studies were also carried out to determine peak asymmetry.

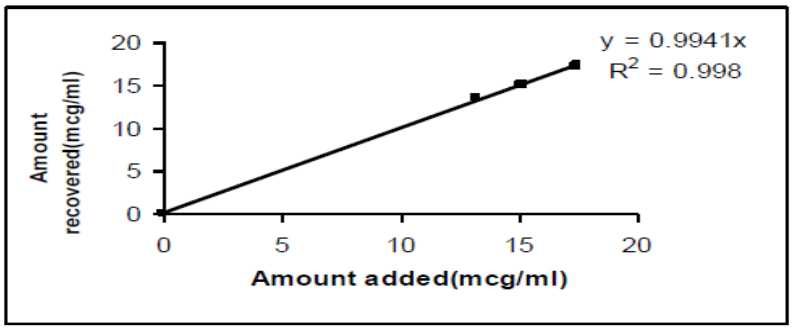


Figure 1: Graphical representation of Accuracy

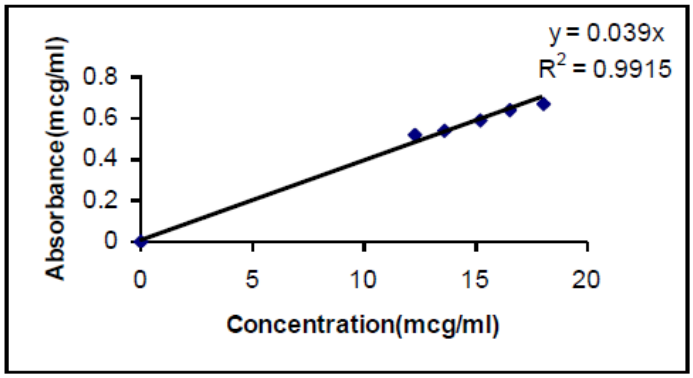


Figure 2: Graphical representation of Linearity

Table 1: Data for System Precision Test

Sample concentration (µg/ml)	No. of Measurements	Absorbance	Relative Standard Deviation
	01	0.607	
	02	0.604	
15.15 (µg/ml)	03	0.600	0.640%

	04	0.608	
	05	0.612	
	06	0.607	

Table 2: Data for Method Precision Test

Sample No.	Sample Weight	Assay (mg) %	Label Claim	Relative Standard Deviation
01	485.78	2.77	105.78	
02	484.70	2.75	105.37	
03	484.68	2.76	105.76	
04	485.58	2.76	106.14	0.271%
05	485.77	2.75	105.38	
06	485.88	2.75	105.34	

Table 3: Data for Accuracy Test

Concentration level	Sample No.	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
	1	13.14	13.44	101.66
80%	2	13.15	13.42	101.56
	3	13.22	13.53	102.14
	1	15.22	14.98	99.91
100%	2	15.24	14.92	98.08
	3	15.08	15.02	99.76
	1	17.28	17.30	99.06
120%	2	17.05	17.06	98.22
	3	17.15	17.14	98.14

Table 4: Data for Linearity Test

Percent Concentration	Concentration (µg/ml/ppm)	Absorbance	Correlation Co-efficient
80	12.55	0.516	
90	13.78	0.524	0.9909
100	15.24	0.546	
110	16.38	0.658	
120	18.09	0.684	

Table 5: Data for Robustness Test

S. No.	Variable Parameters	Assay results
1	Analyst-1	102.53
	Analyst-2	100.28
2	Equipment-1 (UV Spectrophotometer Model-UV-1700)	100.99
	Equipment-2 (UV-Spectrophotometer Model-UV-1601PC)	100.99
3	Day-1	100.68
	Day-2	101.24

From the above data it was observed that all validation parameters (like system suitability, method precision, accuracy, specificity, linearity, robustness) meet the predetermined acceptance criteria. Thus it has been concluded that the method is validated for the analysis of assay of Ofloxacin in Tablet dosage form.

CONCLUSIONS

A new, simple, specific, sensitive, rapid and economical procedure has been developed for determination of Ofloxacin in its dosage form. The objective of this validation of an analytical procedure is to demonstrate that the

drug Ofloxacin is suitable for its intended purpose. The analytical method development recommends the quality, purity and specificity of the drug Ofloxacin tablet form during the manufacturing process and hence the standard of the drug may not vary, which produce the desirable therapeutic effect. The method is based on the ultraviolet absorbance maxima of the above drug at 294nm. The drug obeyed Beer's law in the concentration range of 15µg/ml in methanol. The proposed methods were successfully applied for the determination of drug in commercial tablet preparations. The results of the analysis have been validated statistically and by recovery studies.

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