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Development and Validation of Stability Indicating Assay Method of Ofloxacin in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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Abstract : A simple, precise, sensitive and reproducible stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for determination of Ofloxacin in bulk and pharmaceutical dosage form was developed. The present study describes the development of stability indicating RP-HPLC method for determination of Ofloxacin in presence of its degradation products, generated from forced degradation studies. Ofloxacin drug product were exposed to acid, base, neutral hydrolysis, oxidation, dry heat, photolytic stress conditions and the stressed sample were analyzed by proposed method. The method was carried out on a Qualisil BDS C_{18} (250mm × 4.6mm, 5µm) column with a mobile phase consisting of buffer (0.02 M potassium dihydrogenphosphate): methanol:acetonitrile in ratio 75:15:10 v/v at pH maintained at 3.2 with OPA was used and flow rate of 1 ml/min. The retention time of OFLX was found to be 4.3 min and quantification was achieved with UV detection at 294 nm. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, and specificity, LOD, LOQ, stability in analytical solution etc. The method was found to be accurate with percent recoveries between 97.6 and 92.9 and % RSD was < 2. The above method was a rapid and cost-effective quality-control tool for routine analysis of ofloxacin in bulk and in pharmaceutical dosage form.

Keywords: Ofloxacin, RP-HPLC, stability indicating method, forced degradation studies, validation.

Introduction

Ofloxacin was chemically (RS) 9-fluoro-2, 3dihydro-3-methyl-10-(4methl-1-piperazinyl)-7- oxo-7H-prido [1, 2, 3-de]-1, 4benzoxazine-6-carboxylic acid. Ofloxacin belongs to class of drugs called quinolone antibiotics. Ofloxacin is a broad spectum antibiotic that is active against both Gram-positive and Gram-negitive. It inhibition of topoisomrase enzymes, which inhibits relaxation of supercoild DNA and promotes breakage of double stranded DNA. It is used to treat a variety of bacterial infections[1,2,3].

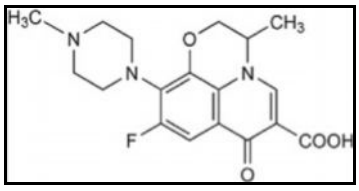

Fig. 1: Structure of ofloxacin

Material and Methods

Chemicals and Reagents

Ofloxacin was gifted by Macleods Pharnaceuticals Limited, Andheri (East), Mumbai. Methanol and acetonitrile of HPLC grade was supplied by Merck Pharmaceuticals. Potassium dihyrogenphosphate of AR grade. All the other reagents were used for analytical grade.

Intrumentation

Agilent technologies HPLC equipped with EZ Chrome elite software, Shimadzu UV probe UV-spectrophotometer, qualisil BDS C_{18} column (250mm × 4.6mm, 5µm),Digital pH meter, Weighing balance AUX-120 Shimadzu, Enertech electronics pvt. Ltd ultrasonicator with UV PDA detector.

Preparation of Phosphate Buffer

Accurately weighed 2.72 gm of potassium dihydrogenphosphate was dissolved in to 500 ml of double distilled water. Volume was made up to 1000 ml with water. The final pH 3.2 was adjusted with *ortho*-phsphoric acid (OPA).

Preparation of Mobile Phase and Dilutions

The mobile phase consisting of buffer (0.02M potassium dihydrogenphosphate): methanol: acetonitrile (pH 3.2 adjusted with OPA) was filtered through 0.20µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 75:15:10 v/v at a flow rate of 1.0 ml/min. The detection was monitored at 294 nm and the run time was 10 min. The volume of injection loop was 20µl [4].

Preparation of Standard Stock Solution

Accurately weighed and transferred 10 mg of Ofloxacin working standard in to the 10 ml volumetric flasks separately. Add 5 ml of diluents, sonicated for 20 minutes and make the final volume with diluents. From the above stock solutions, 0.1 ml was pipette out in to a 10 ml volumetric flask and then make up to the final volume with diluents to concentration 100 µg/ml. Further dilution was made by using this stock solution to obtain final concentrations 20, 40, 60, 80, 100 and 120 µg/ml[5].

Analysis of Marketed Formulation:

To determine the content of Ofloxacin in marketed tablets (label claim 10 mg/ tablet), 20 tablets were weighed, and average weight was calculated. Tablets were triturated and powder equivalent to average weight was weighed. The drug was extracted from the tablet powder with 100 ml methanol. To ensure complete extraction, it was sonicated for 15 min. One ml of supernatant was then diluted up to 10 ml with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. Regression equation, peak area of the sample and the amount of Ofloxacin in the sample was calculated. The amount of Ofloxacin per tablet was thus found[5].

Forced Degradation Studies

A forced degradation study is an important step in drug development process to observe the drug products stability. Forced degradation studies carried out in acid, alkali, oxidative, thermal, photolytic, and hydrolysis degradation[6, 7].

Acid Degradation

Acid degradation was carried out using 20 mg of Ofloxacin in a 250 ml RBF, 20 ml of 1 N hydrochloric acid was added and refluxed for 6 hrs at 40^0 C. The resultant solution 0.1 ml was pipette out in to a 10 ml volumetric flask and then make up to the volume with diluents to obtained concentration 100µg/ml. The resultant solution was injected into the system and the chromatogram was recorded to assess the stability of sample[8].

Alkali degradation

Alkali degradation was carried out using 20 mg of Ofloxacin in a 250 ml RBF, 20 ml of 1 N sodium hydroxide, was added and refluxed for 6 hrs at 40^0 C. The resultant solution 0.1 ml was pipette out in to a 10 ml volumetric flask and then make up to the volume with diluents to obtained concentration 100 µg/ml. The resultant solution was injected into the system and the chromatogram was recorded to assess the stability of sample[9].

Neutral (Water) degradation

Neutral degradation was carried out using 20 mg of Ofloxacin in a 250 ml RBF, 20 ml of water ,was added and refluxed for 8 hrs at 40^0 C. The resultant solution 0.1 ml was pipette out in to a 10 ml volumetric flask and then make up to the volume with diluents to obtained concentration 100µg/ml. The resultant solution was injected into the system and the chromatogram was recorded to assess the stability of sample[10].

Oxidative degradation

Oxidative degradation was carried out using 20 mg of Ofloxacin in 250 ml beaker 20 ml of 6 % H_2O_2 was added and kept for 6 h at room temp. The resultant solution 0.1 ml was pipette out in to a 10 ml volumetric flask and then make up to the volume with diluents to obtained concentration 100µg/ml. The resultant solution was injected into the system and the chromatogram was recorded to assess the stability of sample[10,11].

Thermal degradation:

The standard drug solution was placed in oven at 105^0C for 4 hrs to study dry heat degradation. The resultant solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Method Validation:

Validation is a process which provides high degree of assurance that activity will consistently produce a desired result to meet its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of detection (LOD) and Limit of Quantification (LOQ) as per ICH guideline.

System Suitability Studies

System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions was prepared as per the test method and injected into the chromatographic system. The system suitability parameter was evaluated from tailing factor, retention times and theoretical plates of standard chromatograms.

Standard solution preparations were injected five times into the chromatograph and retention times was recorded. The results obtained are tabulated in Table.

Linearity Study

The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions was Prepared and inject into the HPLC and the chromatograms were recorded[12].

Accuracy

The Accuracy of an analytical method is the closeness of the test results obtained by method to the true value. The study was performed by making three different standard concentrations at 80%, 100% and 1200% levels of known amounts of drug. The accuracy of an analytical method should be established across its range. Finally, the final volume made up with diluents and mixed well. The resulting mixture was analyzed by the proposed HPLC method at 294 nm.

Precision Study

Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The results obtained are tabulated. The retention time and area of six determinations was measured and % RSD was calculated**.** In method precision, a homogenous sample of Ofloxacin was analyzed six times and % RSD was calculated[13].

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that of obtained from the tablet solution .The retention times of the drug standards and the drug from sample solutions were same, so the method was specific without interference from excipients in the tablets[14].

Robustness and Ruggedness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and temperature which may differ but the responses were still within the specified limits of the assay [15].

Results and Discussion

Optimized chromatromatographic conditions:

Various experimental conditions were carried out to achieve the best chromatographic conditions for the determination of the drug substances. Several column types and lengths were tried for better elution and for chromatographic parameters. A good chromatographic separation was achieved with Qualisil BDS C_{18} (250mm × 4.6mm, 5µm), was used for chromatographic separation. The mobile phase composed of 0.02 M Potassium dihydrogenphosphate buffer : acetonitrile : methanol in the ratio (75:15:10v/v), at pH maintained at 3.2 with used OPA; at a flow rate of 1ml/min with run time 10mins.The detection of drugs was carried out at 294nm.The chromatographic condition data was tabulated in table No.1

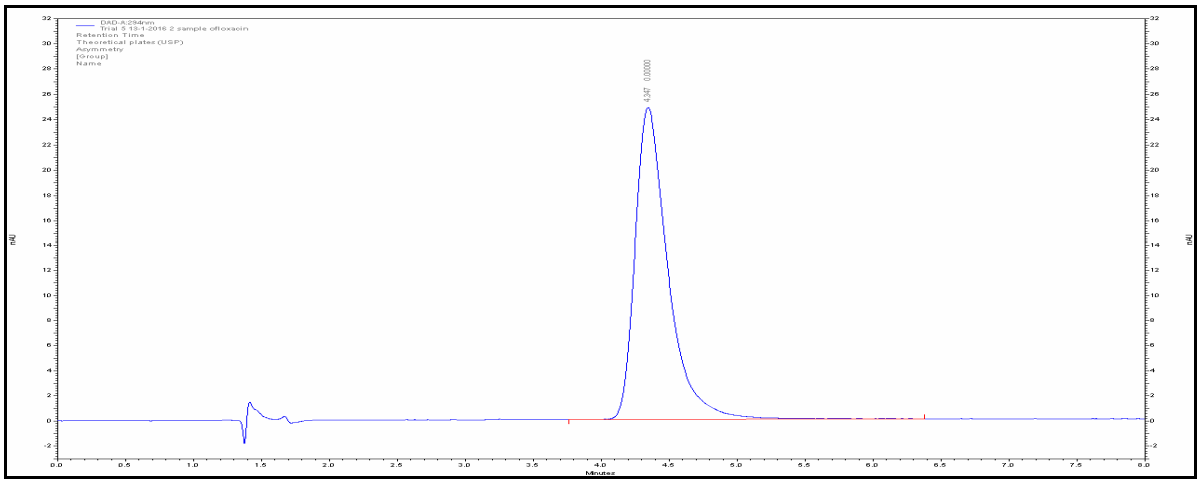

Figure 1. Representative chromatogram of Ofloxacin

Table 1. Optimized chromatographic conditions

Method parameter	Optimized condition
Column	Qualisil BDS C_{18} (250mm × 4.6mm, 5µm)
Wavelength detection	294 nm
Mobile phase composition	0.02 M Potassium dihydrogenphosphate (pH 3.2): methanol: acetonitrile (75:15:10 v/v)
Flow rate	1.0 ml/min
Run time	10 mins

Forced degradations studies:

Summary of degradation product was tabulated in table No.2.

Table 2. Summary of degradation products of ofloxacin

Condition	Time (in hrs)	Drug peak area at zero hrs	Drug peak area stressed sample	Retention time of degradant (mins)	% Degradation
1 N HCL	6	4340207	4049554	2.4,4.10	6.6
1 NaOH	6	4399017	4049215	4.1,7.2	7.9
Water	8	4346490	4127842	4.2,7.0	5.03
H_2O_2, 6%	6	420908	3953633	2.5,4.1&7.2	7.8
Dry heat 105^0C	4	4400786	4276747	4.2	2.8

Acidic degradation: Ofloxacin showed sufficient degradation at 6 hrs, in 1 N HCL. The degradation product formed for OFLX was at retention time (Rt) 4.10 min. It has shown in fig.2

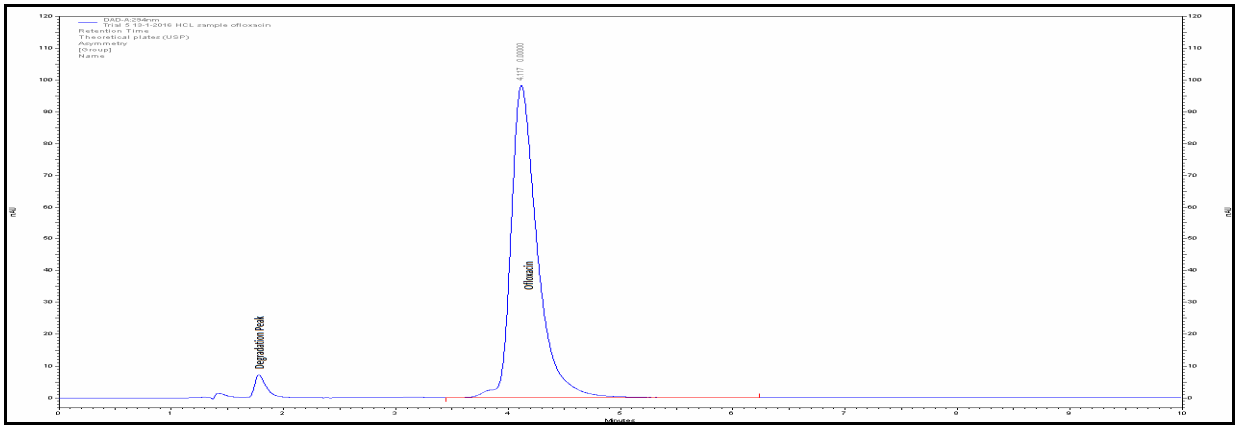

Figure 2. Representative chromatogram of acid treated ofloxacin (1N HCl, refluxed for 6 h)

Alkali degradation: The drug shown sufficient degradation in alkaline hydrolysis in 1 N NaOH at 40^0 C. The degraded product appeared at Rt 4.10 min for OFLX. It has shown in fig.3.

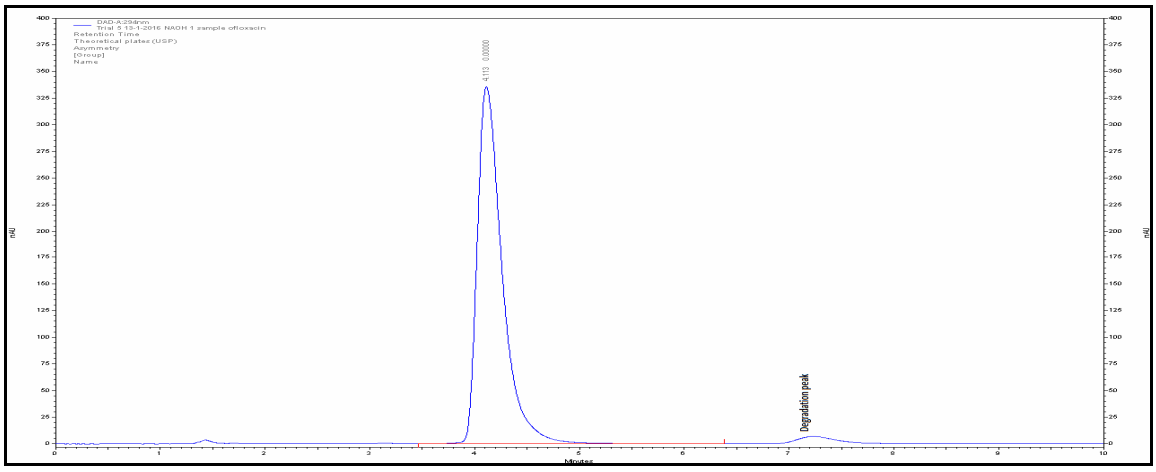

Figure 3. Representative Chromatogram of alkali degradation of Ofloxacin.

Neutral (Water) degradation: The degradation product of ofloxacin was found at 8 hrs. at 40^0 C. It has shown in fig.4.

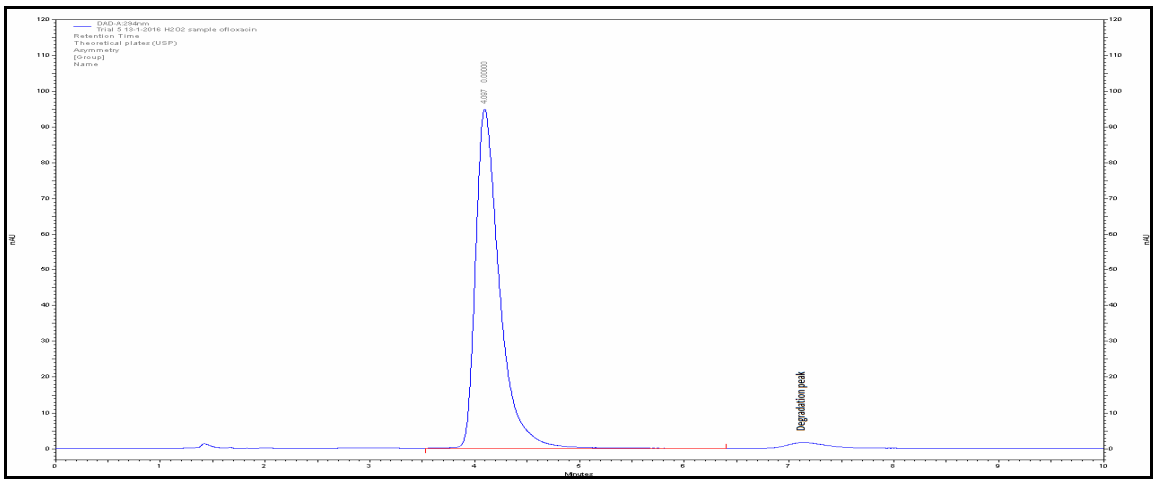

Figure 4. Representative chromatogram showing water treated Ofloxacin

Oxidative degradation: The drug Ofloxacin sufficient degradation in 6 % H2O2 for 6 hrs at room temperature. It has shown in fig.5.

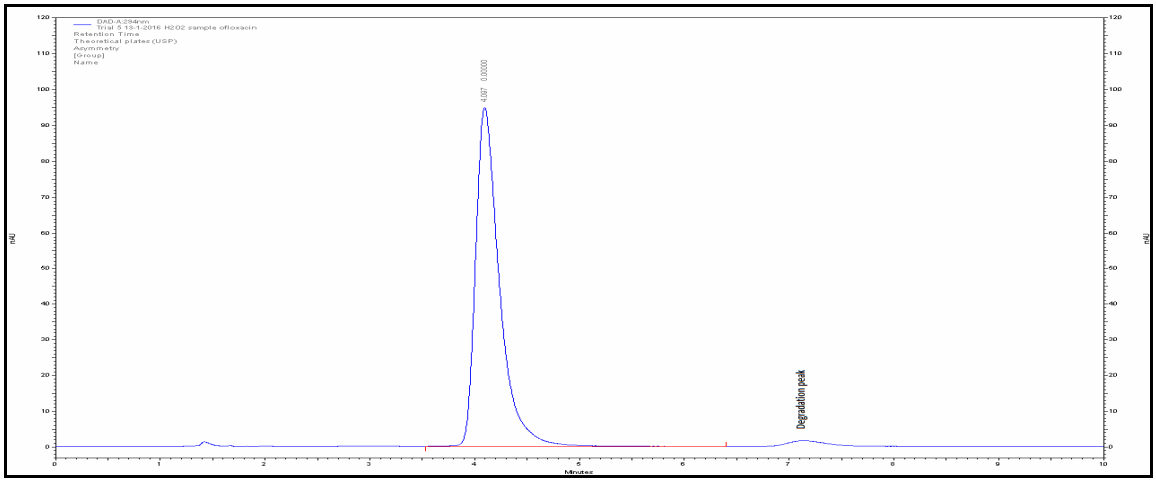

Figure 5. Representative chromatogram of Oxidative degradation study of Ofloxacin

Thermal degradation: The thermal degradation product was in oven at 105^0 C for 4 hrs at Rt 4.2 min. It has shown in fig.6.

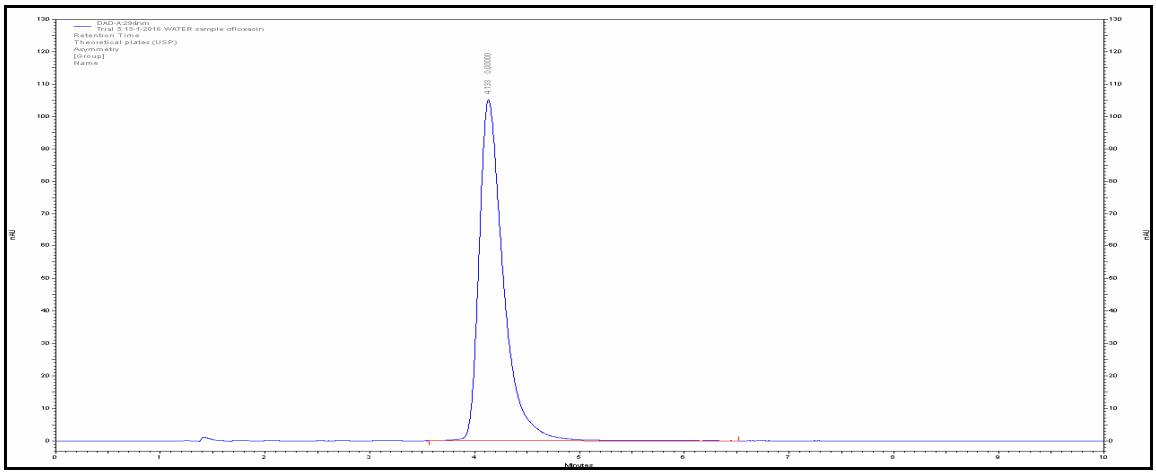

Figure 6. Representative chromatogram of dry heat degradation of Ofloxacin

Validation of the developed stability-indicating method

The developed stability indicating method was validated according to ICH guidelines. The validation parameters addressed were linearity, precision (inter-day, intra-day and intermediate precision), accuracy and LOD, LOQ, robustness.

Linearity: From the Linearity data it was observed that the method was showing linearity in the concentration range of 20-120µg/ml at 294 nm for Ofloxacin. Correlation coefficient (R^2) was found to be 0.999 for the compound. The linearity data was tabulated in Table.3. The Chromatograms for the linearity data were shown in the fig no: and the linearity curve was plotted and given in the Fig.7

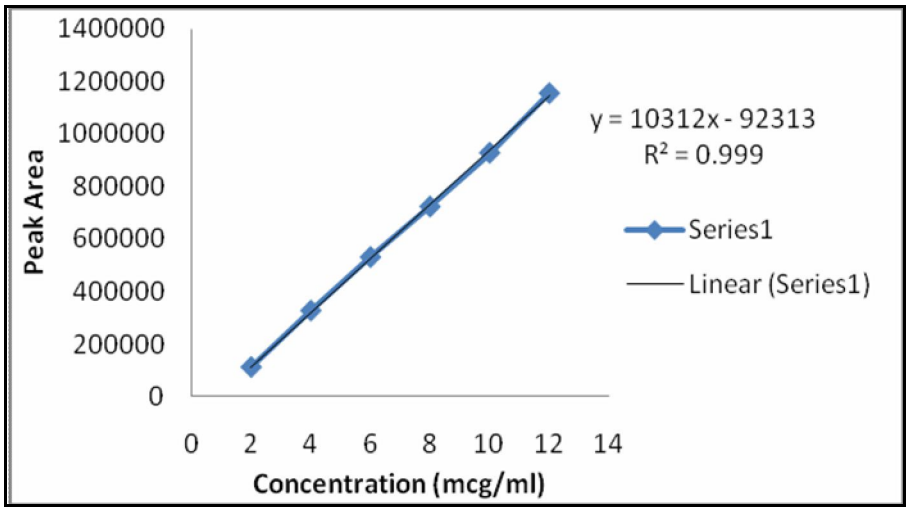

Figure 7. Calibration curve of ofloxacin

Table 3. Linearity data for Ofloxacin

Calibration standard	Nominal Concentration µg/ml	Peak Area of replicate (µV.sec)			Mean peak area	Standard deviation of peak area	% RSD
		1	2	3			
1	2	110486	112586	111687	111586.333	1053.613	0.94421329
2	4	325248	332549	322750	326849	5091.9054	1.55787701
3	6	527635	527536	537637	530936	5803.4473	1.09305968
4	8	735465	709566	725667	723566	13076.705	1.80725801
5	10	918545	938646	928978	928723	10052.926	1.0824461
6	12	1145665	1175667	1145687	1155673	17315.315	1.49828848
Equation	**Y = 10312X + 92313 r^2= 0.999**						

Specificity: The Chromatograms of Standard and Sample are identical with nearly same Retention time. There is no interference with blank and placebo to the drugs. The chromatograms were shown in the Figures 1 for standard, sample, blank and placebo.

Accuracy: Recovery studies of the drugs were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the preanalysed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 80, 100 and 120% levels. The percentage recovery and its % RSD were calculated. The method was found to be accurate with percent recoveries between 97.6 and 92.9 and % RSD was < 2. The results were tabulated in the Table.4.

Table 4. Accuracy data of Ofloxacin

% level of recovery	Initial Amount	Amount Added	Area	Amount found	% Amount found	Amount Recovery	% Recovery
80 %	4	3.2	642511	7.1259	97.6847	3.1259	97.6847
	4	3.2	641247	7.1137	97.3017	3.1137	97.3017
	4	3.2	642511	7.1259	97.6847	3.1259	97.6847
			Mean	7.1218	97.5571	3.1218	97.5571
			SD	0.0071	0.2212	0.0071	0.2212
			%RSD	0.0994	0.2267	0.2267	0.2267
100%	4	4	703513	7.7175	92.9369	3.7175	92.9369
	4	4	703715	7.7194	92.9858	3.7194	92.9858
	4	4	703922	7.7214	93.0360	3.7214	93.0360
			Mean	7.7194	92.9862	3.7194	92.9862
			SD	0.0020	0.0496	0.0020	0.0496
			%RSD	0.0257	0.0533	0.0533	0.0533
120%	4	4.8	773864	8.3997	91.6604	4.3997	91.6604
	4	4.8	774065	8.4016	91.7010	4.4016	91.7010
	4	4.8	774169	8.4027	91.7220	4.4027	91.7220
			Mean	8.4013	91.6945	4.4013	91.6945
			SD	0.0015	0.0313	0.0015	0.0313
			%RSD	0.0179	0.0342	0.0342	0.0342

Precision: The %RSD for the sample chromatograms of method precision were found to be Intra- day 0.9975 .& Interday 1.46 for Ofloxacin . Hence it passes method precision. The results were tabulated in the Table.6.

Ruggedness: Comparison of both the results obtained for two different Analysts shows that the method was rugged for Analyst-Analyst variability. The system suitability parameters of Ruggedness were found to be within the limits and were tabulated in Table.5. The Chromatograms for ruggedness were shown in Figures 1.

Table 5. Ruggedness data of Ofloxacin

	Sr. No.	Concentration	Area	Amount found	% Amt. found
Analyst 1	1	6	523452.0	5.971344065	99.5224011
	2	6	524362	5.980168735	99.6694789
	3	6	524325	5.97980993	99.6634988
Analyst 2	4	6	524351	5.980062064	99.6677011
	5	6	524345	5.980003879	99.6667313
	6	6	524361	5.980159038	99.6693173
			Average	5.978591285	99.6431881
			SD		0.05921341
			% RSD		0.05942545

Robustness: There was no significant change in the retention time of Ofloxacin and its degradation products after introducing small changes in mobile phase composition indicating robustness of the method.

Analysis of Marketed Formulation: The chromatograms of the drug samples extracted from tablets did not show a change in the retention time. There was no interference from the excipients, which are commonly present in the tablets. The drug content was found to be 99.66 % with a % RSD of 0.2003 as shown in (**Table 6)** Therefore it may be concluded that, degradation of Ofloxacin had not occurred in the marketed formulations. The % low RSD value indicated the suitability of the method for the routine analysis of Ofloxacin in pharmaceutical formulation.

Table 6. Summery of Validated Parameters of Ofloxacin

Parameters	Ofloxacin
Calibration range(µg/ml)	2-12 µg/ml
Correlation coefficient(R^2)	0.999
Precision(Intra-day)%RSD	0.9975
Precision(Inter-day)%RSD	1.46
Retention time	4.3
LOD	0.0484
LOQ	0.1466
Assay	97.68%

Conclusion

Stability indicating reversed phase high performance liquid chromatography method for Ofloxacin is developed and validated as per ICH guidelines. The analysis of ofloxacin in the presence of degradants is possible now. From this study it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Ofloxacin in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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