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Original Article

Hydrolytic Degradation Kinetic Study of Balofloxacin by Stability Indicating Reversed Phase High Performance Liquid Chromatography Method

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ABSTRACT

Background: Balofloxacin is a third generation fluoroquinolone with a broad antibacterial spectrum ranging from gram-positive bacteria to gram-negative bacteria. It is used in treatment of uncomplicated urinary tract infections. No stability indicating analytical method has been reported for BFX. Also stress degradation studies of Balofloxacin were not found in literature. **Objective:** To develop and validate a stability indicating RP-HPLC method for estimation of Balofloxacin in presence of its hydrolytic degradation products. **Materials and Method:** The chromatographic separation was performed using C_{18}, Grace Smart column (250 x 4.6 mm), 5 µm as the stationary phase and Water: Acetonitrile: Tri ethylamine (72:28:1 v/v/v), pH adjusted to 3.0 using ortho-phosphoric acid as mobile phase with detection wavelength 294 nm. The developed method was validated according to ICH Q2R1 guideline. Balofloxacin was subjected to degradation in acidic, alkaline and neutral conditions. **Results and Discussion:** The linearity was established over concentration range of 20-100 µg/ml with correlation coefficient r^2 = 0.9979. Recovery of drug was achieved in the range of 99.19–101.65%. Limit of Detection and Limit of Quantitation was found to be 4.13 and 12.51 µg/ml. Balofloxacin was found to be stable under alkaline and neutral conditions, while it degraded under acidic hydrolytic condition. The retention times for Balofloxacin and its acid degradation product were found to be 7.0 ± 0.1 and 5.7 ± 0.1 minutes, respectively. **Application:** The developed RP-HPLC method was applied for estimation of Balofloxacin in its tablet dosage forms and results were found to be in good agreement with the labeled claim. The method was also applied for degradation kinetic study of Balofloxacin in acidic medium. **Conclusion:** The developed RP-HPLC method was found to be accurate, precise, specific and sensitive. It can be applied for routine analysis (assay) of tablets containing Balofloxacin. The degradation of Balofloxacin in all conditions was found to be first order and highest degradation was found in 2.0 N HCl at 75°C.

Key words: Stability indicating HPLC method, Balofloxacin (BFX), Degradation products, Degradation kinetic study, Design expert software–9.

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INTRODUCTION

Balofloxacin is a third generation fluoroquinolone with a broad antibacterial spectrum ranging from gram-positive bacteria to gram-negative bacteria. BFX exhibits excellent antibacterial activity against gram positive bacteria such as multiple-drug-resistant staphylococci and pneumococci.[1] Chemically it is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylamino-piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid[2] (Figure 1). BFX is a chemotherapeutic bactericidal which eradicates bacteria by interfering with DNA replication. It is used in treatment of uncomplicated urinary tract infections.

Degradation study of drug itself and its pharmaceutical formulation allows a better knowledge of its therapeutic, physicochemical and toxicological behaviour. The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products and also helps in deciding the routes of administration and storage conditions of various pharmaceutical dosage forms. Thus it is helpful to produce quality, safe and efficacious dosage forms.[3-8] Hydrolysis is one of the prominent routes of degradation of drugs containing functional groups like ester, amide, carboxylic acid, etc. Water, either as a solvent or in the form of moisture, contacts most pharmaceutical dosage forms to some degree. The potential for this degradation pathway exists for most drugs and excipients. It is a known fact that hydrolytic degradation products may be formed due to acidic environment in the formulation in presence of acidic excipients. Extensive literature review reveals that several spectrophotometric, spectrofluorimetric and RP-HPLC methods have been reported for estimation of Balofloxacin in its pharmaceutical dosage forms.[9-22] No stability indicating analytical method has been reported for BFX. Also stress degradation studies of Balofloxacin were not found in literature. Therefore it was thought of interest to develop and validate stability indicating RP-HPLC methods for estimation of Balofloxacin in its marketed dosage forms. The developed method was applied to study hydrolytic degradation kinetics of BFX in acidic medium at different temperature and thereby to determine rate constant, half life and order of reaction. The developed method was also applied to determine the presence of degradation products in marketed formulations stored at 50°C for six months.

EXPERIMENTAL

Stability indicating HPLC method for Balofloxacin Instrumentation

The HPLC system (LC-10AT, Shimadzu) consisting of SPD-10A UV detector and C_{18}, Grace Smart column (250×4.6 mm, 5 µm), syringe (25 µl capacity, Hamilton) and 0.45 micron nylon millipore filter were used for degradation kinetic study of BFX and analysis of its marketed formulations. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

Chemicals and reagents

Balofloxacin was obtained as a gift sample from a reputed pharmaceutical company. HPLC grade acetonitrile, triethyl amine, ortho phosphoric acid, acetic acid and hydrochloric acid AR were purchased from s.d. Fine-Chem Limited, Mumbai, India. Double distilled water was prepared in the laboratory. Bazucin, Baloforce, Balotero and B Cin tablets were purchased from local market.

Chromatographic conditions

Standard and sample solutions of BFX were injected in the column using 25 µl micro syringe. The chromatogram was run for appropriate time using mobile phase, Water: Acetonitrile: Tri ethylamine (72:28:1 v/v/v) pH adjusted to 3.0 using ortho-phosphoric acid, which was previously degassed. Detection was carried out at wavelength 294 nm. The chromatogram was stopped after complete separation was achieved. Data of peak like area, height, retention time, resolution were recorded using Clarity software. System suitability tests include resolution (RS), capacity factor (*k'*), number of theoretical plates (N) and tailing factor (Tf) which were determined for API and degradation product peaks.

Preparation of solutions

Preparation of Working Standard Solution of BFX

Accurately weighed quantity of BFX (100 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 5% acetic acid to give a stock solution having strength of 1 mg/ml. Stock solution (10.0 ml) was transferred into 100 ml volumetric flask and diluted up to the mark with 5% acetic acid solution to give 100 ***µg***/ml of BFX.

Preparation of sample solution for forced degradation study of BFX

Forced degradation of BFX was carried out under acidic, alkaline and neutral conditions.

Acidic hydrolysis

Accurately weighed quantity of BFX (100 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 2.0 N HCl to give a stock solution having strength of 1 mg/ml. The flask was placed in water bath at 80 ± 2°C for 3 h. The solution (1.0 ml) was withdrawn and transferred to 10 ml volumetric flask, cooled to room temperature, volume was made up to the mark with mobile phase (100 µg/ml) and 20µl was injected into the column.

Alkaline hydrolysis

Accurately weighed quantity of BFX (100 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 2.0 N NaOH to give a stock solution having strength of 1 mg/ml. The flask was placed in water bath at 80 ± 2°C for 3 h. The solution (1.0 ml) was withdrawn and transferred to 10 ml volumetric flask, cooled to room temperature, volume was made up to the mark with mobile phase (100 µg/ml) and 20 µl was injected into the column.

Neutral condition

Accurately weighed quantity of BFX (100 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with distilled water to give a stock solution having strength of 1 mg/ml. The flask was placed in water bath at 80 ± 2°C for 3 h. The solution (1.0 ml) was withdrawn and transferred to 10 ml volumetric flask, cooled to room temperature, volume was made up to the mark with mobile phase (100 µg/ml) and 20 µl was injected into the column.

Procedure for Calibration Curve

The series consisted of different concentrations of BFX solution ranging from 20-100 µg/ml. The solutions were prepared by pipetting out 2.0, 4.0, 6.0, 8.0 and 10.0 ml of the working standard solution of BFX (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with mobile phase. Solutions were injected and analyzed by HPLC as described under chromatographic conditions. The graph of peak area versus respective concentration was plotted.

Method Validation

Specificity

Specificity of the method was determined by analyzing a blank solution, a placebo containing common pharmaceutical excipients and acid degraded sample solution.

Linearity

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 20-100 µg/ml for BFX (n=5). The calibration curve of peak area vs. respective concentration was plotted and correlation coefficient and regression line equation was computed.

Precision

I. Repeatability

Aliquots of 6.0 ml of working standard solution of BFX (100 µg/ml) were transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with mobile phase to get 60 µg/ml of BFX and injected seven times to system and analyzed as described under chromatographic conditions. The peak area of the solutions was measured and % CV was calculated.

II. Intraday precision

Solutions containing 20, 40, 60, 80 and 100 µg/ml BFX were analyzed 3 times on the same day using HPLC and % CV were calculated.

III. Inter day precision

Solutions containing 20, 40, 60, 80 and 100 µg/ml BFX were analyzed on 3 different days using HPLC and % CV were calculated.

Accuracy

It was determined by calculating the recovery of BFX from tablet formulation by standard addition method. Into a pre-analyzed sample of BFX tablets, an increasing amount of BFX standard solution was spiked to get three different concentration levels, 80, 100 and 120%. Each solution was prepared in triplicate, analyzed and percent of BFX recovered was calculated.

Limit of Detection and limit of Quantitation

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows.

$$LOD = 3.3 * SD/slope\ of\ calibration\ curve$$

$$LOQ = 10 * SD/slope\ of\ calibration\ curve$$

$$SD = Standard\ deviation\ of\ intercept.$$

Procedure for assay of marketed formulations

Twenty tablets were weighed accurately and powdered. The quantity of tablet powder equivalent to 100 mg BFX was transferred into 100 ml volumetric flask dissolved and diluted up to mark with 5% acetic acid. The solution was filtered through Whatman filter paper; 4.0 ml of filtrate was transferred into 100 ml volumetric flask and diluted up to mark with mobile phase to get 40 µg/ml solution which was analyzed as described under chromatographic conditions.

Determination of degradation products in marketed tablets

Strip packs of four brands of BFX tablets were placed in a petri-dish in an oven at 50°C. At the end of 6 months, tablets were analyzed by the developed method as described under chromatographic conditions.

Procedure for degradation kinetic study of BFX in acidic medium

Accurately weighed quantity of BFX (100 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 0.1 N HCl. The flask was placed in a thermostatically controlled water bath for 8 h at temperature 25°C/50°C/75°C. At each one hour interval, 1.0 ml of solution was pipetted out in 10 ml volumetric flask, cooled to room temperature and diluted up to mark with mobile phase. The solution was analyzed by HPLC as described under chromatographic conditions. Similar procedure was followed using 1.0 N and 2.0 N HCl solutions. Area of drug peak was noted and concentration was calculated using calibration curve straight line equation. From this, order of reaction, degradation rate constant and half life were calculated. Contour plots were constructed using design expert software for parameters like degradation rate constant, half life and % drug degraded at 8 h. Parameters like degradation rate constant, shelf life and half life were predicted at 30°C, 37°C and 42°C temperatures, which are the average room temperatures observed in India in different seasons and different places.

RESULTS AND DISCUSSION

Stability indicating HPLC method for estimation of BFX

Optimization of Mobile Phase

The combination of water: acetonitrile: tri ethyl amine (70: 30: 1 v/v/v) at pH 3.0 using o-phosphoric acid provided optimum polarity for proper migration, separation and resolution of BFX. However changing the mobile phase composition to water: acetonitrile: tri ethyl amine (72: 28: 1 v/v/v) [pH 3.0 using o-phosphoric acid] improved the value of tailing factor for BFX from 1.54 to 1.25, without significant change in retention times and resolution. Therefore it was selected as mobile phase for estimation of BFX in presence of its acid degradation product.

Selection of wavelength for measurement

The mode of detection was absorption in the UV region. The wavelength of maximum absorption was found to be 294.0 nm. This wavelength was selected as detection wavelength in HPLC (Figure 2).

Analysis of Forced Degradation Samples of BFX

BFX was initially treated with 2.0 N HCl, 2.0 N NaOH and water by placing BFX solutions in water bath at 80 ± 2°C for 3 h. BFX, after alkaline and neutral treatment, did not show any peak other than BFX peak. This result indicates that BFX is highly stable in alkaline and neutral condition. However, on treatment with 2.0 N HCl, BFX was degraded and one additional peak was obtained other than BFX peak. Retention times for BFX and its acid degradation product were 7.0 $\pm$ 0.1 and 5.7 $\pm$ 0.1 min respectively (Figure 3).

Method Validation

System suitability testing

Capacity factor for BFX, tailing factor for BFX, average number of plates in the column and resolution between BFX and its degradation product are shown in Table 1.

Specificity

The blank and placebo solution did not show any peaks and degradation product peak was found to appear at retention time different from that of the drug.

Linearity and calibration curve

The linearity range for BFX was found to be in the range of 20-100 µg/ml. Correlation co-efficient for calibration curve of BFX was found to be 0.9979. The regression line equation for BFX is as following, y=62.97x+873.4. Correlation coefficient is >0.997, indicates linearity of method within the given range (Figure 4 and 5).

Precision

The % C.V. of repeatability for BFX was found to be 0.48. The % C.V. of intra-day precision and inter-day precision for BFX were found to be 0.43-0.80% and 0.53-1.02 % respectively.

Accuracy

Accuracy was determined in terms of percentage recovery. The recoveries were performed at three levels i.e. 80%, 100% and 120%. The % recovery for BFX was found to be 99.19-101.65 %.

Limit of detection and Quantitation

The LOD and LOQ for BFX were found to be 4.13 and 12.51 µg/ml respectively.

The summary of validation parameters is shown in Table 2.

Assay of market formulations of BFX

The proposed method was applied for assay the tablet dosage forms containing BFX and % amount of BFX was found to be 98.45-102.02 % of labelled claim of BFX tablets. Results are shown in Table 4. The chromatogram of BFX from marketed formulation showed no additional peak except BFX indicate that excipients and other additives used in formulation did not interfere in assay of BFX (Figure 6). Marketed formulations stored at 50 ± 2°C were analyzed after 6 months. Assay results are presented in Table 3 which showed that there were no acid degradation products and formulations were stable.

Degradation kinetic study of BFX in acidic condition

The acidic degradation kinetic study of BFX was performed in 0.1, 1.0 and 2.0 N HCl solutions at 25, 50 and 75°C.

Effect of strength of hydrochloric acid on degradation of BFX:

At 25°C, as the strength of HCl was increased from 0.1 N to 1.0 N, BFX showed 1.6 times increase in degradation rate constant. The degradation rate constant was 2.2 times higher in 2.0 N HCl as compared to that in 0.1 N HCl, while it was 1.4 times higher than that in 1.0 N HCl. At 50°C, as the strength of HCl was increased from 0.1 N to 1.0 N, BFX showed 1.5 times increase in degradation rate constant. The degradation rate constant was 1.9 times higher in 2.0 N HCl as compared to that in 0.1 N HCl, while it was 1.3 times higher than that in 1.0 N HCl. At 75°C, as the strength of HCl was increased from 0.1 N to 1.0 N, BFX showed 1.3 times increase in degradation rate constant. The degradation rate constant was 1.7 times higher in 2.0 N HCl as compared to that in 0.1 N HCl, while it was 1.3 times higher than that in 1.0 N HCl. Increase in acid strength does affect the degradation of BFX which was clear from the fact that degradation rate constants at all three temperatures increased as the strength of acid was increased (Figure 7).

Effect of temperature on degradation of BFX

In 0.1 N HCl, as the temperature was increased from 25°C to 50°C, BFX showed 1.4 times increase in degradation rate constant. The degradation rate constant was 2 times higher at 75°C as compared to that at 25°C, while it was 1.5 times higher than that at 50°C. In 1.0 N HCl, as the

Table 1: System suitability data for BFX

System suitability test		Observed value
Resolution (Rs)	Between BFX and D.P.	2.03
Capacity factor (k') (for BFX)		4.07
Number of Plates (N avg)		11524
Tailing factor (for BFX)		1.25

Table 2: Summary of validation parameters

Parameters	Result
Linearity Range	20-100 µg/ml
Correlation Coefficient	0.9979
Precision (%CV)	
Repeatability (n=7)	0.48
Inter day precision (n=3)	0.53-1.02
Intraday precision (n=3)	0.43-0.80
Accuracy (% Recovery)	99.19-101.65%
LOD (µg/ml)	4.13
LOQ (µg/ml)	12.51
Specificity	Specific

Table 3: Results for assay of marketed formulations

Formulation	Label claim (mg)	Assay (% label claim) (n=3)	
		Initial	After 6 months
Tablets (B-1)	100 mg	100.97	97.54
Tablets (B-2)	100 mg	98.45	97.15
Tablets (B-3)	100 mg	99.23	96.78
Tablets (B-4)	100 mg	102.02	98.54

Table 4: Summary of degradation kinetic study of BFX in acidic medium

HCl (N)	Temp. (°C)	Rate constant ($Kx10^{-4}$)		Half life (min)	Shelf life (min)	% drug deg. at 8 h.	Activation energy (Kcal/mole)	
		Average	From graph				From eqn.	From graph
0.1	25	7.09	6.98	993.11	150.47	28	2.92	2.89
	50	10.0	9.83	704.71	106.77	38		
	75	14.18	14.12	490.88	74.38	49		
1.0	25	11.19	11.11	624.3	94.59	41	2.11	2.10
	50	14.78	14.32	483.77	73.3	49		
	75	18.28	18.52	374.27	56.71	59		
2.0	25	15.22	15.43	449.12	68.05	52	1.84	1.83
	50	18.10	18.45	375.67	56.92	59		
	75	23.55	24.11	287.4	43.55	70		

Table 5: Prediction from contour plot

(A) At temperature 25°C

HCl (N)	K x 10^{-4} (min^{-1})	Half life (min)	Shelf Life (min)
0.1	6.462	941.79	142.69
0.3	7.411	886.51	134.32
1.2	11.683	637.73	96.62
2.0	15.479	416.59	63.12

(B) At temperature 30°C

HCl (N)	K x 10^{-4} (min^{-1})	Half life (min)	Shelf Life (min)
0.1	7.236	894.76	135.57
0.7	10.084	739.56	112.05
1.6	14.355	506.75	76.78
2.0	16.254	403.29	61.10

(C) At temperature 37°C

HCl (N)	K x 10^{-4} (min^{-1})	Half life (min)	Shelf Life (min)
0.1	8.320	828.91	125.59
0.5	10.219	735.38	111.42
1.2	13.541	571.71	86.62
2.0	17.338	384.66	58.28

(D) At temperature 42°C

HCl (N)	K x 10^{-4} (min^{-1})	Half life (min)	Shelf Life (min)
0.1	9.095	781.87	118.46
0.7	11.942	652.23	98.82
1.4	15.265	500.99	75.91
2.0	18.112	371.36	56.27

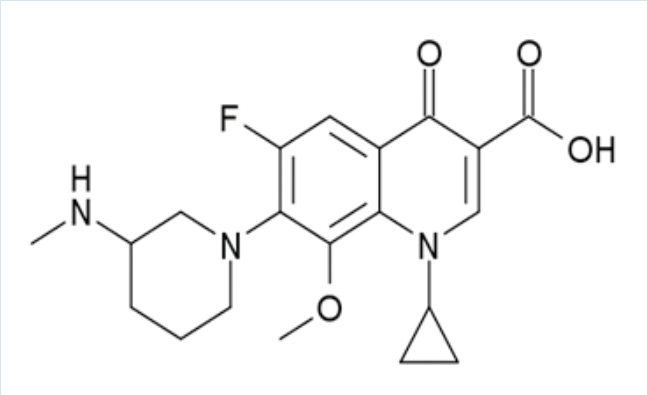


Figure 1: Chemical structure of Balofloxacin.

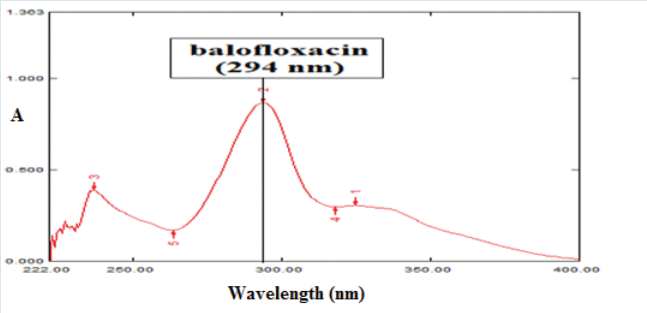
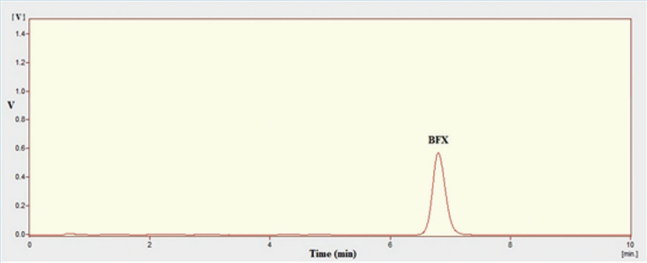
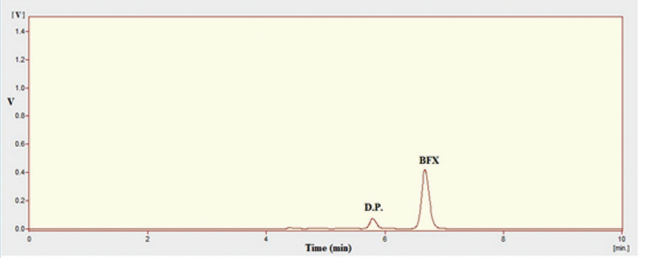


Figure 2: UV spectrum of standard BFX showing selection of wavelength for measurement.



(A)



(B)

Figure 3: Chromatogram of (a) Standard BFX (b) Acid treated BFX.

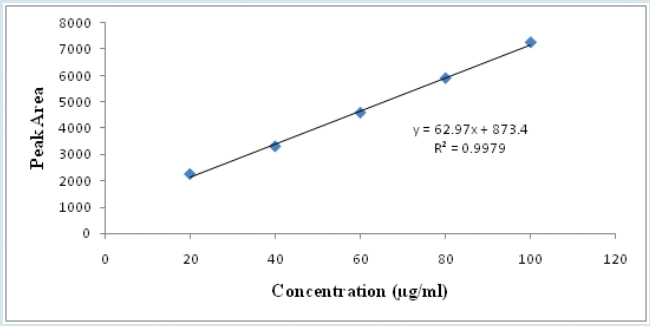


Figure 4: Calibration curve for BFX at 294 nm (20-100 µg/ml).

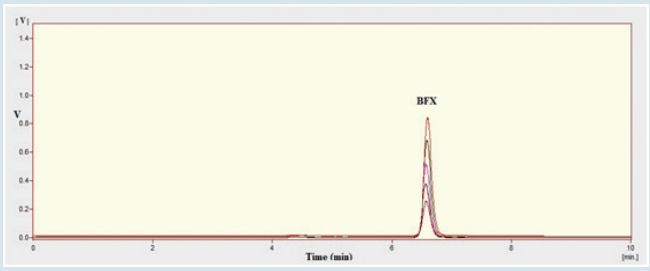


Figure 5: Overlain chromatograms of calibration data for BFX (20-100 µg/ml).

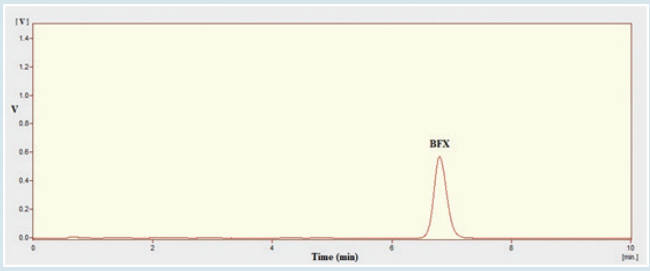


Figure 6: Chromatogram showing the BFX sample from dosage form.

temperature was increased from 25°C to 50°C, BFX showed 1.3 times increase in degradation rate constant. The degradation rate constant was 1.7 times higher at 75°C as compared to that at 25°C, while it was 1.3 times higher than that at 50°C. In 2.0 N HCl, as the temperature was increased from 25°C to 50°C, BFX showed 1.2 times increase in degradation rate constant. The degradation rate constant was 1.6 times higher at 75°C as compared to that at 25°C, while it was 1.3 times higher than that at 50°C. Rise in temperature does affect the degradation of BFX which was clear from the fact that degradation rate constants in all three strengths of HCl increased as temperature was increased (Figure 8).

The rate of hydrolysis increased, while half life decreased with increase in strength of acid as well as temperature (Figure 9 and 10). The plots of log C versus time for all conditions were found to be linear indicating first order degradation kinetics.

Energy of Activation (Ea):

A plot of ln k as a function of 1/T referred as Arrhenius plot is linear if Ea is independent of temperature. The slope of line obtained from plot of ln k versus 1/T is equal to–Ea/R.

$$-Ea/R = slope$$

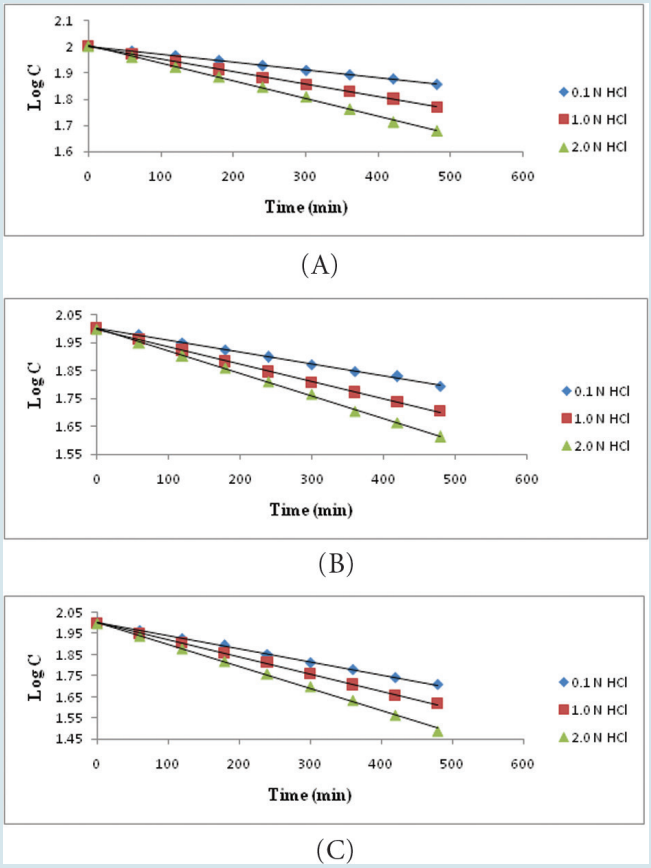


Figure 7: Comparison of degradation of BFX in 0.1 N, 1.0 N and 2.0 N HCl at (a) 25°C (b) 50°C (c) 75°C.

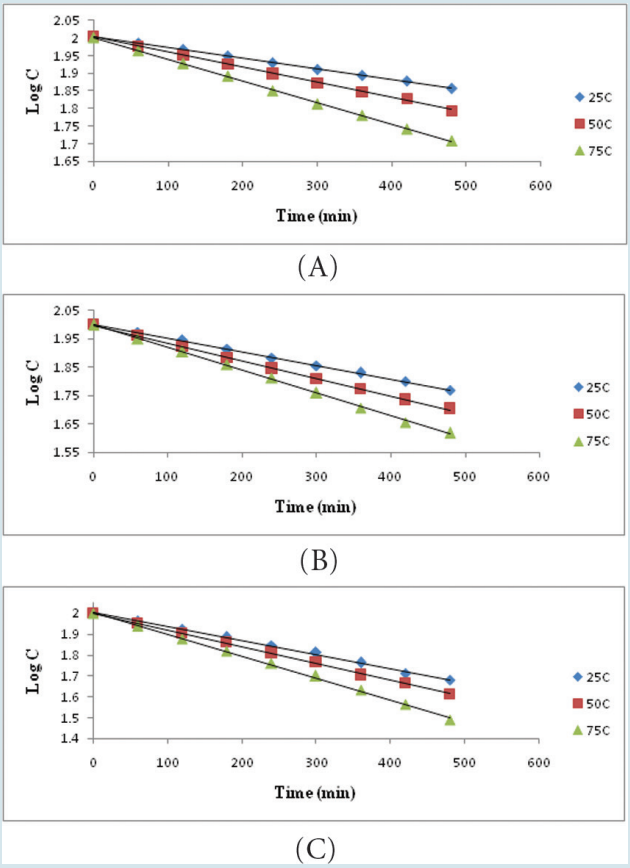


Figure 8: Comparison of degradation of BFX at 25°C, 50°C and 75°C in (a) 0.1 N (b) 1.0 N and (c) 2.0 N HCl.

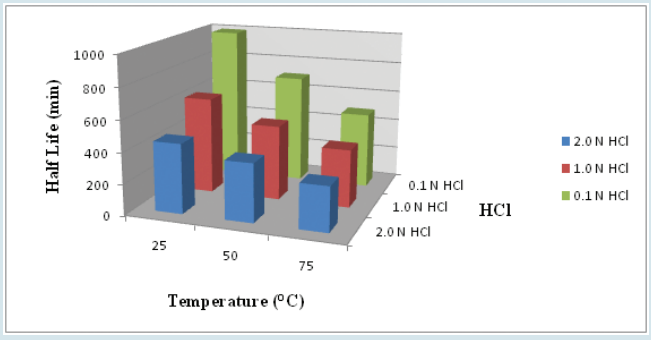


Figure 9: Comparison of half-life of BFX in acidic medium.

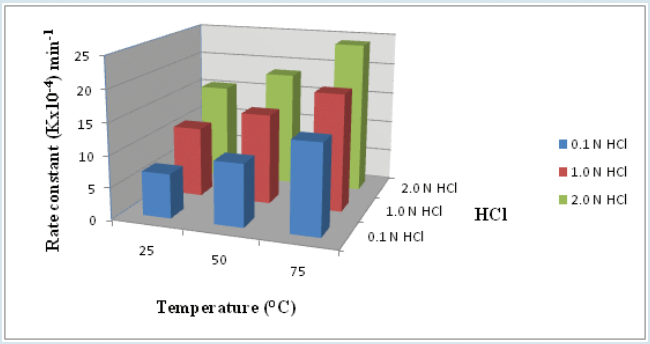


Figure 10: Comparison of degradation rate constant of BFX in acidic medium.

The activation energy (Ea) of the acidic degradation process of BFX in different strengths of HCl was calculated from Arrhenius plot and is shown in Figure 11.

Summary of degradation kinetic study of BFX in acidic medium is shown in Table 4.

Prediction of degradation rate constant using design expert software-9:

The whole design was performed using 2 factor 3 level design.

Factor: HCl strength; Level: 0.1 N, 1.0 N, 2.0 N

Factor: Temperature; Level: 25°C, 50°C, 75°C

Contour plots were constructed using design expert software for parameters like degradation rate constant, half life and % drug degraded at 8 h. These contour plots can be applied to predict degradation. Parameters like degradation rate constant, shelf life and half life were predicted at 25°C, 30°C, 37°C and 42°C temperature (Table 5), which are the

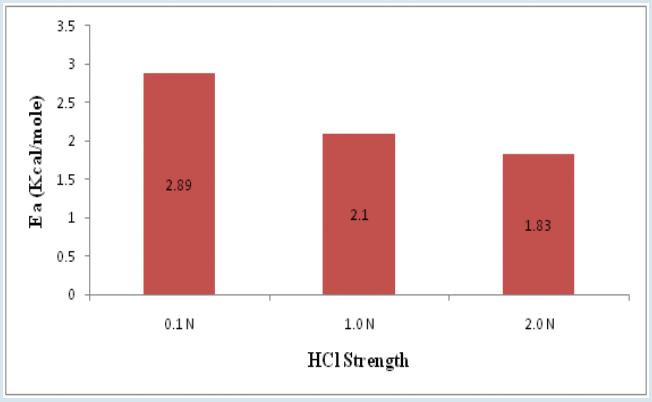


Figure 11: Activation energies for degradation of BFX in 0.1 N, 1.0 N and 2.0 N HCl.

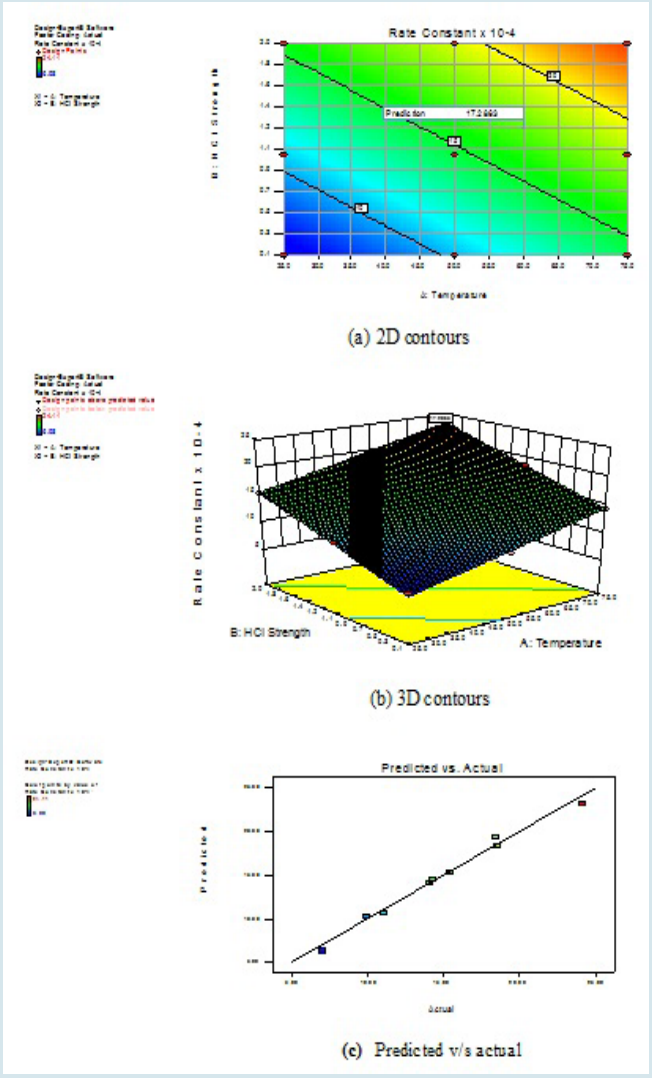


Figure 12: Plots with respect to rate constants (a) 2D contours (b) 3D contours.

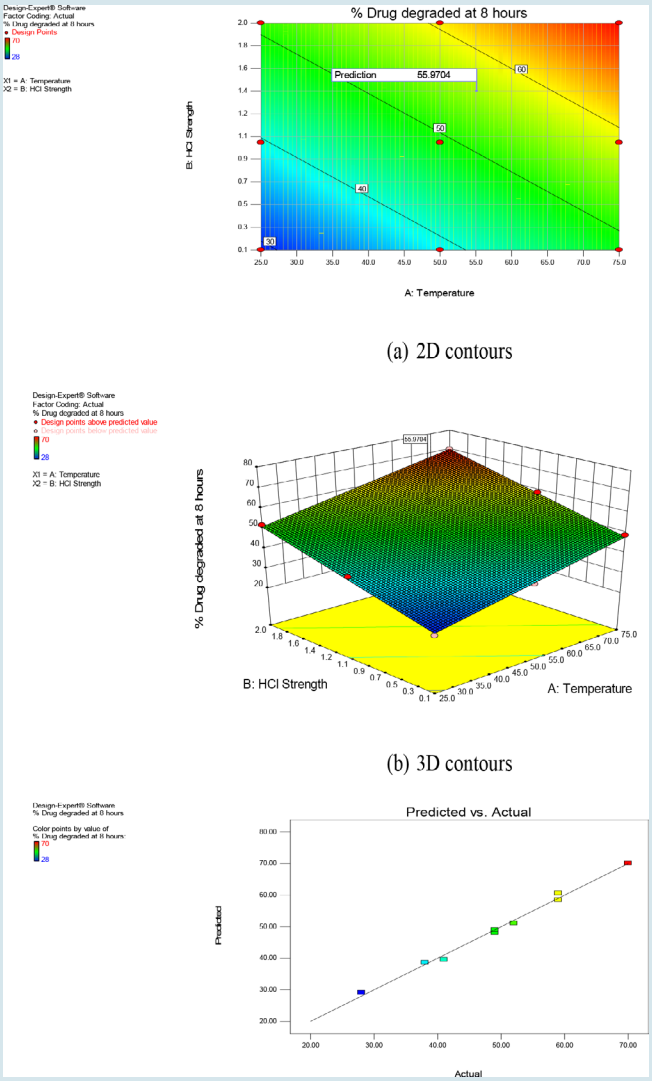


Figure 13: Plots with respect to percent drug degraded at 8 h (a) 2D contours (b) 3D contours.

average room temperatures observed in India in different seasons and different places. Contour plots are shown in Figure 12, 13 and 14.

Future Implications

The developed method can be further optimized to study degradation of BFX in oxidative, photolytic and dry heat conditions. Also the degradation products can be isolated and characterized to understand the mechanism of degradation. The behavior of BFX when exposed to hydrolytic stress conditions was found to be similar to other fluoroquinolones i.e. stable in alkaline and neutral conditions and degraded in acidic conditions. The acidic degradation product of BFX was not characterized in the present study but based on degradation behaviour of other fluoroquinolones in similar condition; it could be a de-carboxylated (at position 3) derivative of BFX.

CONCLUSION

Stability indicating RP-HPLC method has been developed and validated using C_{18}, Grace Smart column (250×4.6 mm 5 µm) as the stationary phase and Water: Acetonitrile: Tri ethylamine (72:28:1 v/v/v), pH adjusted to 3.0 using ortho-phosphoric acid as mobile phase with detection

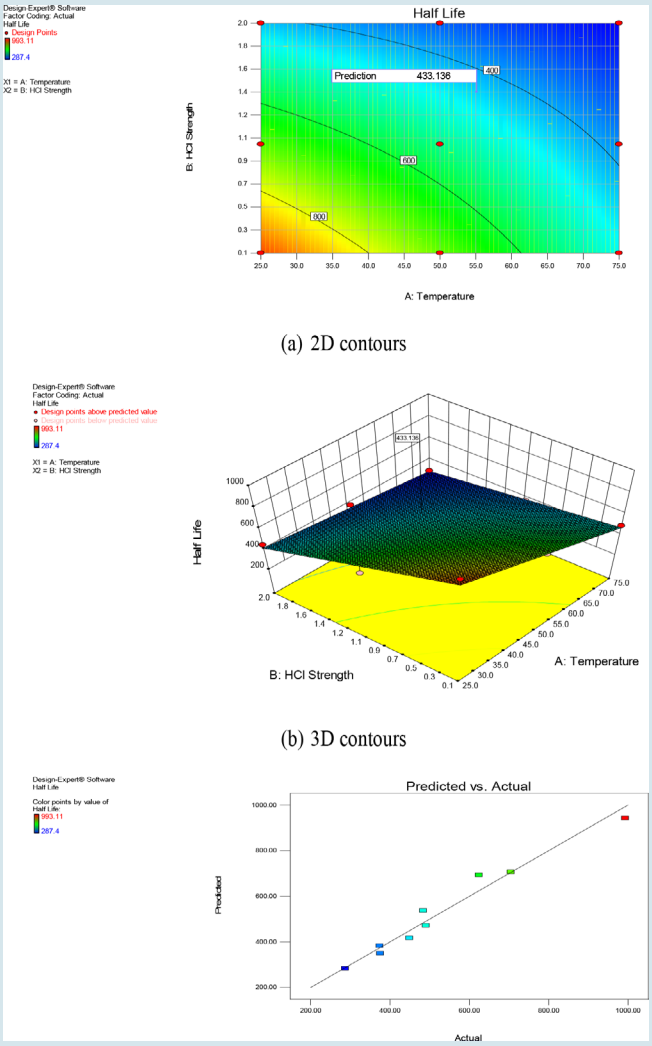


Figure 14: Plots with respect to half life (a) 2D contours (b) 3D contours (c) Predicted v/s actual.

wavelength 294 nm. The developed method was able to quantify BFX in presence of its acidic degradation product, excipients and additives. So, developed method was specific and stability indicating for estimation of BFX. The developed method was applied for assay of pharmaceutical dosage forms of BFX and assay results found were in good agreement with labeled claim of dosage forms. The developed method was also applied for degradation kinetic study of BFX in acidic medium. The degradation of BFX in all conditions was found to be first order and highest degradation was found in 2.0 N HCl at 75^{0}C.

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CONFLICT OF INTEREST

None declared.

ABBREVIATION USED

BFX: Balofloxacin, **LOD:** Limit of Detection, **LOQ:** Limit of Quantitation, **RP-HPLC:** Reversed Phase High Performance Liquid Chromatography, **UV:** Ultra violet, **CV:** Co-efficient of Variance, **SD:** Standard Deviation.

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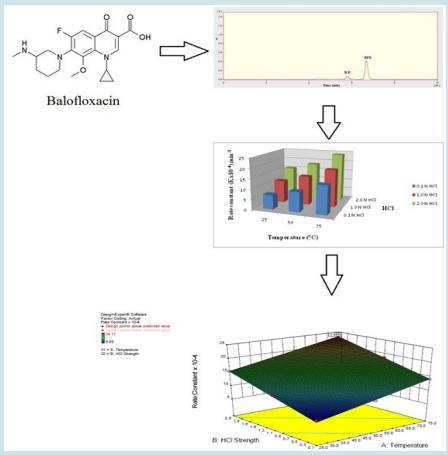
PICTORIAL ABSTRACT



SUMMARY

- Stability indicating HPLC method was developed and validated as per ICH (Q2R1) guidelines for estimation of Balofloxacin in presence of its hydrolytic degradation products.
- Balofloxacin was subjected to degradation in acidic, alkaline and neutral conditions.
- It was found to be stable under alkaline and neutral conditions, while it degraded under acidic hydrolytic condition.
- The proposed method was also applied for degradation kinetic study of Balofloxacin in 0.1N, 1.0N and 2.0N hydrochloric acid at different temperatures i.e. 25°C, 50°C and 75°C.

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