AAPS PharmSciTech, Vol. 15, No. 6, December 2014 (© 2014)
DOI: 10.1208/s12249-014-0184-x

Research Article

Photodegradation of Moxifloxacin in Aqueous and Organic Solvents: A Kinetic Study

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Received 28 January 2014; accepted 23 July 2014; published online 20 August 2014

Abstract. The kinetics of photodegradation of moxifloxacin (MF) in aqueous solution (pH 2.0–12.0), and organic solvents has been studied. MF photodegradation is a specific acid-base catalyzed reaction and follows first-order kinetics. The apparent first-order rate constants (k_{obs}) for the photodegradation of MF range from 0.69×10^{-4} (pH 7.5) to 19.50×10^{-4} min^{-1} (pH 12.0), and in organic solvents from 1.24×10^{-4} (1-butanol) to 2.04×10^{-4} min^{-1} (acetonitrile). The second-order rate constant (k_2) for the $[H^+]$-catalyzed and $[OH^-]$-catalyzed reactions are 6.61×10^{-2} and 19.20×10^{-2} M^{-1} min^{-1}, respectively. This indicates that the specific base-catalyzed reaction is about three-fold faster than that of the specific acid-catalyzed reaction probably as a result of the rapid cleavage of diazabicyclononane side chain in the molecule. The k_{obs}-pH profile for the degradation reactions is a V-shaped curve indicating specific acid-base catalysis. The minimum rate of photodegradation at pH 7–8 is due to the presence of zwitterionic species. There is a linear relation between k_{obs} and the dielectric constant and an inverse relation between k_{obs} and the viscosity of the solvent. Some photodegraded products of MF have been identified and pathways proposed for their formation in acid and alkaline solutions.

KEY WORDS: acid-base catalysis; kinetics; moxifloxacin; photodegradation; rate–pH profile; solvent effect.

INTRODUCTION

Moxifloxacin (MF) (1-cyclopropyl-6-fluoro-8-methoxy-7-((4*aS*,7*aS*)-octahydro-6H-pyrrolo [3,4-*b*] pyridin-6-yl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (Fig. 1)) is a synthetic, broad spectrum fluoroquinolone antimicrobial agent (1–3) that shows *in vitro* activity against Gram-positive and Gram-negative organisms, as well as atypical organisms and anaerobes (4,5). It is indicated for the treatment of mild to moderate community-acquired pneumonia including that is caused by multidrug-resistant *Streptococcus pneumoniae*, acute bacterial exacerbation of chronic sinusitis, complicated dermatological and intraabdominal infections, bacterial conjunctivitis, and as a second line agent in tuberculosis (6–8). It is available in different dosage forms such as tablets, injections, and eye drops (9). Fluoroquinolones are involved in the photosensitized degradation of DNA (10) and also exhibit cellular phototoxicity (11). They are sensitive to light (10,12–15), and several studies have been carried out on the photodegradation of MF (11,16–19) and other fluoroquinolones (18,20–28). The photodegradation of quinolones (12) and fluoroquinolones in aqueous solution (17–19,24,28), follows first-order kinetics.

Chromatographic methods including high-performance liquid chromatography (HPLC) (18,28–35), ultra-performance liquid chromatography (UPLC) (19) and densitometric high-performance thin-layer chromatography (HPTLC) (36) have been used for the assay of MF and detection of its chemical and photodegradation products (19,29,33,34,36,37). Some photodegradation products of MF in aqueous solution have been identified by HPLC, NMR, and MS studies (18,19,21,22). In the present investigation, the kinetics of photodegradation of MF has been studied over a wide range of pH by HPLC to observe its degradation behavior with a change in ionic species and whether the reaction undergoes any acid-base catalysis. The effect of solvent dielectric constant and viscosity on the rate of photodegradation of MF in aqueous and organic solvents has also been studied to throw light on the mode of its degradation reactions. The chemical structure of MF is shown in Fig. 1.

MATERIALS AND METHODS

Materials

MF (98%) was obtained from Bayer AG (Leverkusen, Germany) and was used without further purification. Acetonitrile and methanol (HPLC grade) and all other solvents and reagents (Analytical grade) were purchased from Merck & Co., Whitehouse Station, NJ, USA. Deionized water was used

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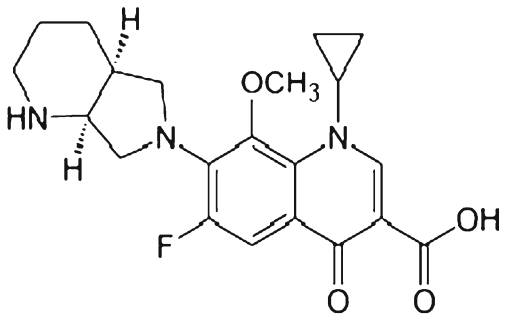


Fig. 1. Chemical structure of MF

throughout the study obtained from Millipore Milli-Q plus system (Bedford, USA). The solvents and solutions were filtered through a Millipore filtration unit and degassed before use. The following buffer systems were used in the study: KCl–HCl (pH 1.0–2.0); citric acid–Na_2HPO_4 (pH 2.5–8.0); $Na_2B_4O_7$–HCl (pH 8.5–9.0); $Na_2B_4O_7$–NaOH (pH 9.5–10.5); Na_2HPO_4–NaOH (pH 11.0–12.0); the ionic strength was set as 0.02 M in each case.

Precaution

The experiments were performed in a dark chamber under subdued light. Freshly prepared solutions of MF, protected from light, were used each time in order to avoid the effect of any chemical or photochemical change.

HPLC Assay

Chromatographic Conditions

The HPLC system (model LC-10ATVP, Shimadzu, Japan) was equipped with a UV-detector (model SPD-10AVP) that was connected to a microcomputer system. The analytical column used was Purospher RP-8 endcapped (5 μm). The HPLC analysis was carried out at room temperature (25±1°C) using isocratic condition. The mobile phase consisted of a mixture of water and acetonitrile (50:50, *v*/*v*) with 0.3% triethylamine at pH 3.3 adjusted with phosphoric acid. The volume of injection was 20 μL and the flow rate was 1.0 ml min^{-1}. All the solutions and the mobile phase were sonicated for 20–25 min before use. The detection of MF was carried out at 290 nm. The method was validated under the condition used before its application to the assay of the drug in photodegraded solutions.

Method Validation

The validation of the HPLC method was carried out according to International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidelines with respect to parameters including system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

System Suitability

System suitability was performed by injecting six replicates of standard solutions of moxifloxacin at 10 μg/ml before sample analysis. It involved the equilibration of the composition of mobile phase. The acceptance criterion of relative standard deviation (%RSD) for peak area was less than 2%. Column plates greater than 700 and tailing factor according to USP was less than 2.0.

Table I. Validation Data for MF

System suitability[a]	
Retention time (min)±SD	2.133±0.002
%RSD	1.058
Resolution	–
Theoretical plate	2571
Tailing factor	1.57
Linearity range (μg/ml)	3.0–24.0
Correlation coefficient	0.9997
Slope	59,914.65
Intercept	15,044.13
SE[b] of intercept	6,525.466
SD[c] of intercept	14,591.39
Recovery range (%)[d]	100.45–101.20
Accuracy (%)[e] ±SD[c]	101.08±0.584
Precision[a]	
Intraday	
Recovery range (%)	99.00–101.58
%RSD	0.372–0.775
Interday	
Recovery range (%)	100.09–100.69
%RSD	0.040–0.469
LOD (μg/ml)	0.80
LOQ (μg/ml)	2.44
Robustness[a]	
Flow rate (ml/min)	1.0±0.1
Retention time	1.984–2.068
%RSD	0.026–0.1973
pH of mobile phase	3.3±0.1
Retention time	1.869–2.024
%RSD	0.024–0.028
Ratio of mobile phase[f]	50:50±2.0
Retention time	1.955–2.121
%RSD	0.025–0.280

[a] $n=5$
[b] SE standard error
[c] SD standard deviation
[d] Recovery (%)=(amount found/amount added)×100, where amount found was calculated from (mean area of five determinations−intercept)/slope (Ahmed *et al.*, 2013)
[e] Accuracy (%)=Mean recovery range
[f] Water:acetonitrile=48:52, 50:50, 52:48

Linearity

Eight standard solutions in the concentration range 3–24 μg/ml (3, 6, 9, 12, 15, 18, 21, and 24 μg/ml) were injected into the chromatograph and calibration curve was constructed by plotting the peak area against concentration in micrograms per milliliter.

Accuracy and Precision

The precision of the method was established by injecting five samples at three different concentration levels for the intra- and interday precision in duplicate. The accuracy and

precision were expressed as %recovery range and %RSD of the analyte.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated from calibration curve using the following equation:

$$\mathrm{LOD} = 3.3 \times \sigma/S$$
$$\mathrm{LOQ} = 10 \times \sigma/S$$

where σ is the standard deviation and S is the slope of the standard curve.

Robustness

Robustness of the method was determined by a small change in ratios of the mobile phase (±2: ±2), flow rate (±0.1 ml min^{-1}) and pH (±0.1 unit).

Photolysis

A 5×10^{-5} M aqueous solution of MF (100 ml) was prepared in appropriate buffer in the pH range of 2.0–12.0 in a 100 ml beaker (Pyrex, France), and immersed in a water bath maintained at 25±1°C. The solution was irradiated with a Philips 30 W TUV tube (Netherlands) (87% emission at 290 nm) in a dark chamber. The tube was fixed horizontally at a distance of 25 cm from the center of the vessel. Samples were withdrawn at appropriate intervals for chromatographic assay. The same procedure was applied to the photolysis of MF (5×10^{-5} M) in various organic solvents and the samples were assayed by HPLC method.

For the identification of photodegraded products formed in acid and alkaline solutions, 1 mg/ml solution of MF was irradiated under the same conditions for 24–30 h and the solutions were used for LC–MS/MS analysis.

Light Intensity Measurement

Potassium ferrioxalate actinometery was performed (38) for the measurement of the intensity of Philips 30 W TUV tube and a value of $5.56\pm0.12\times10^{18}$ quanta s^{-1} was obtained.

LC–ESI–QqTOF–MS/MS Analysis of MF and Photodegraded Products

The MF standard and its photodegraded products in acidic and alkaline media were diluted in water and working dilution was prepared in 50:50 acetonitrile–water containing 0.1% formic acid (HCOOH). Analysis was performed by

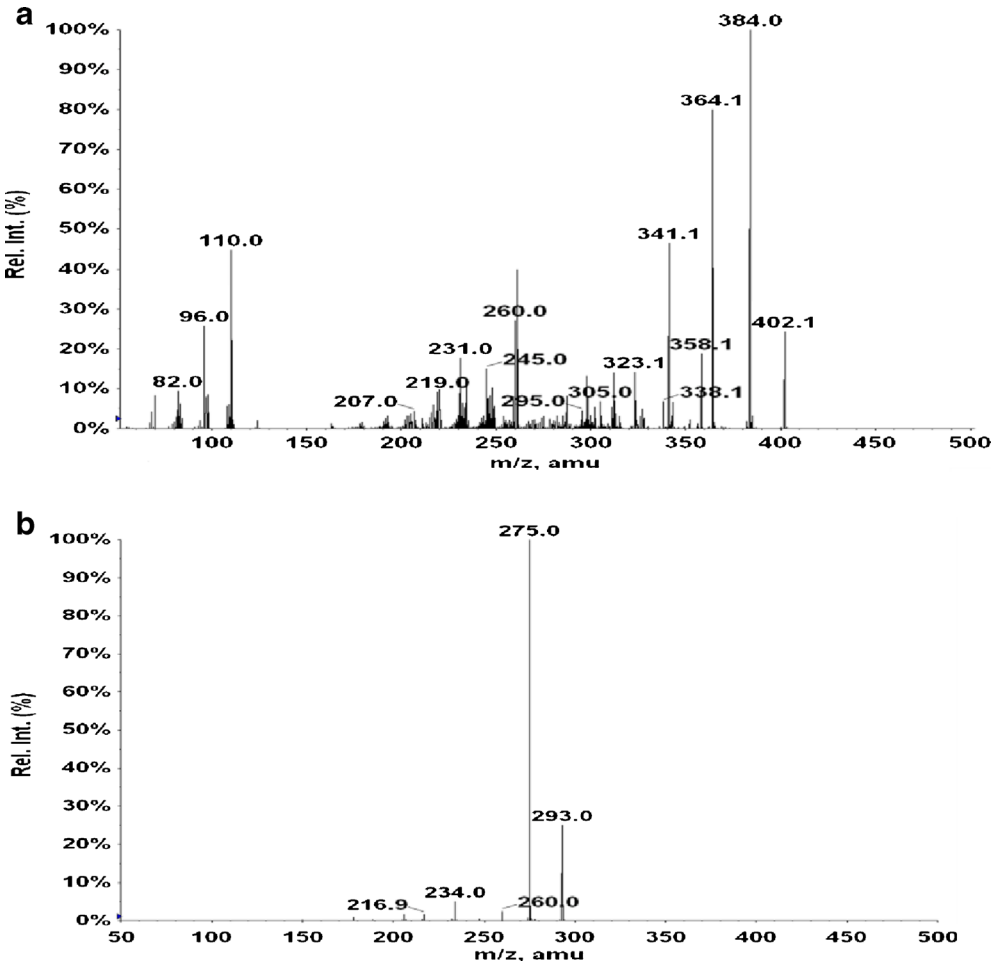


Fig. 2. Product ion spectra of MF (**a**) and its photodegraded product MP-4 (**b**), at collision energy of 25 eV

electrospray ionization (ESI) and collision-induced dissociation (CID), positive ion mode, on Qq–TOF–MS/MS instrument (QSTAR XL mass spectrometer Applied Biosystem/MDS Sciex, Darmstadt, Germany) coupled with 1100 HPLC system (Agilent). Chromatographic separation was performed using a HPLC system (Series 1100, Agilent Technologies, Waldbronn, Germany), and reversed-phase column (ZORBAX XDB-C18, 50×4.6 mm, 1.8 mm, 600 Bar, Agilent). Injection volumes were 5 µl. The mobile phases were as follows: eluent A, H_2O (0.1% formic acid) and eluent B, ACN (0.1% formic acid), properly filtered and degassed for 15 min in ultrasonic bath before use. The flow rate was 0.5 ml min^{-1} and a gradient elution program was used. The chromatographic procedure was initialized at 5% B, raised to 100% B at 10 min, returning to initial conditions to 5% B at 12 min.

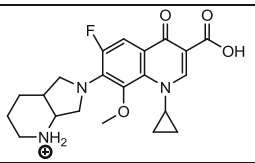
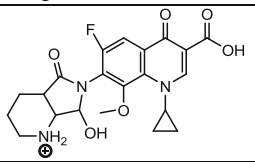
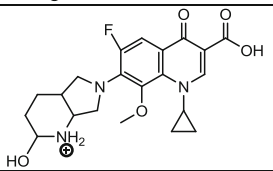
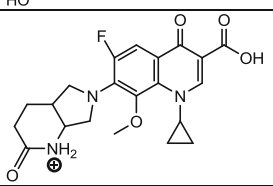
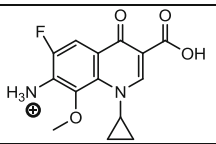
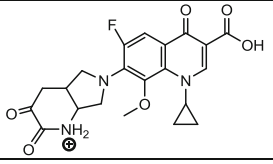
High-purity nitrogen gas was used as the curtain gas and collision gas delivered from Peak Scientific nitrogen generator. The ESI interface conditions were as follows: ion spray capillary voltage of 5,500 V, curtain gas flow rate 20 L min^{-1}, nebulizer gas flow rate 30 L min^{-1}, DP1 60 V, DP2 10 V, and focusing potential of 265 V. The collision energy was swept from 20 to 45 eV for MS/MS analysis. The sample was introduced into the mass spectrometer using a Harvard syringe pump (Holliston, MA) at a flow rate of 5 µl min^{-1}.

RESULTS AND DISCUSSION

Method Validation

Validation of an analytical method is of utmost importance for accurate and precise estimation of the analyte (39). Therefore, in the present study, a simple, accurate and sensitive HPLC method has been validated prior to its application to the assay of MF. The validation data of MF are reported in Table I. The results indicate that the current method is highly accurate (101%) and precise (%RSD<2%) for the determination of MF with better system suitability (Table I). The method has also shown good linearity (R^2=0.9997) in the studied range of 3.0–24.0 µg/ml with the minimum detection and quantification limits of 0.80 and 2.44 µg/ml, respectively. Small and deliberate changes (robustness) have not been shown to affect the accuracy and precision of the method thus indicating

Table II. Photodegraded Products of MF

Product ID	Proposed formula	Observed mass	Calculated mass	Error (ppm)	Proposed structure
MF	$C_{21}H_{25}FN_3O_4^+$	402.1803	402.1829	–6.4892	
MP–1[a,b]	$C_{21}H_{23}FN_3O_6^+$	432.1574	432.1570	0.7195	
MP–2[a]	$C_{21}H_{25}FN_3O_5^+$	418.1755	418.1778	–5.5586	
MP–3[a]	$C_{21}H_{23}FN_3O_5^+$	416.1702	416.1621	19.2844	
MP–4[a,b]	$C_{14}H_{14}FN_2O_4^+$	293.0914	293.0937	–8.0534	
MP–5[a,b]	$C_{21}H_{21}FN_3O_6^+$	430.150	430.1414	19.9024	

[a] Found in acid
[b] Found in base

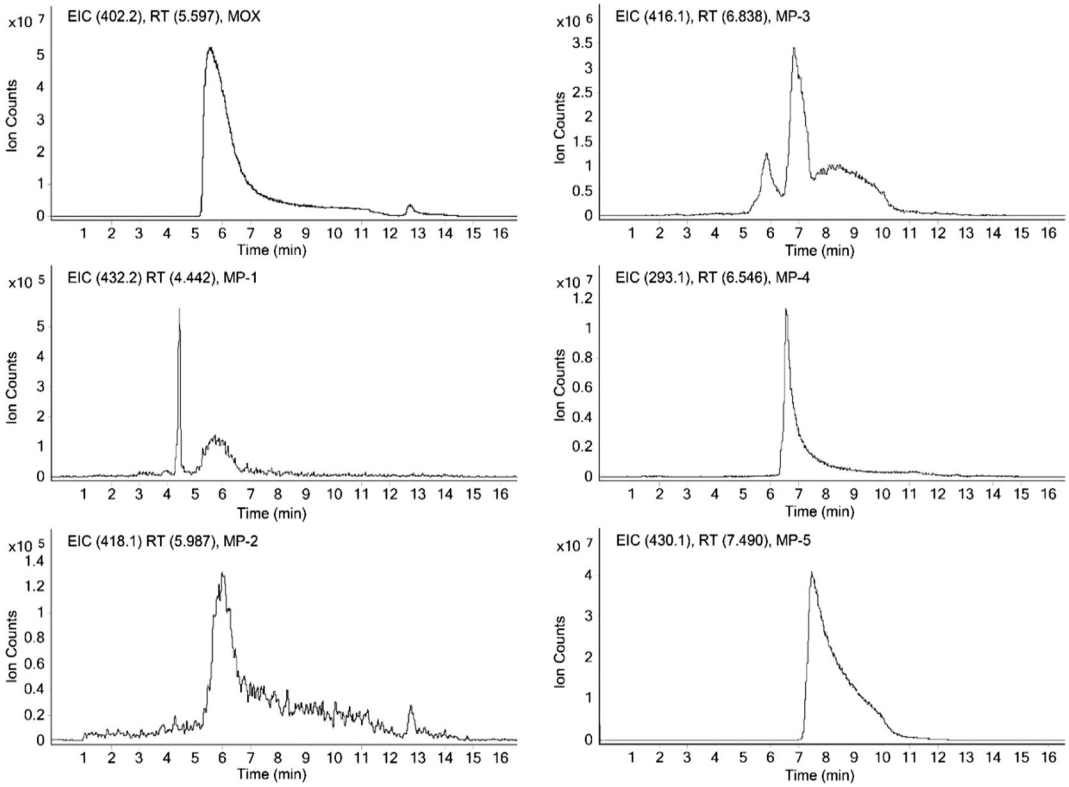


Fig. 3. Extracted-ion chromatogram (EIC) of MF and its photodegraded products

that the current method can be successfully applied to the assay of MF and its photoproducts.

Identification of Photodegraded Products

The photodegraded products of MF under acidic and alkaline conditions (pH 2.0–12.0) obtained on 24–30 h irradiation were identified on the basis of LC–ESI–QTOF–MS and MS/MS analysis. ESI–QqTOF–MS (positive mode) scan of MF and its degradation products in acidic and alkaline solutions showed peak $[M+H]^+$ at *m/z* 402.1803 (MF), 432.1574 (MP-1), 418.1755 (MP-2), 416.1702 (MP-3), 293.0914 (MP-4) and 430.150 (MP-5), corresponding to the molecular formulae $C_{21}H_{25}FN_3O_4^+$ (calc. 402.1829), $C_{21}H_{23}FN_3O_6^+$ (calc. 432.1570), $C_{21}H_{25}FN_3O_5^+$ (calc. 418.1778), $C_{21}H_{23}FN_3O_5^+$ (calc. 416.1621), $C_{14}H_{14}FN_2O_4^+$ (calc. 293.0937) and $C_{21}H_{21}FN_3O_6^+$ (calc. 430.1414), respectively. MP-4 was found to be a major degraded product in both acidic and alkaline conditions. MS/MS spectra of standard MF and its major degraded product are provided in Fig. 2. The identified products are previously known (18,19). Proposed structure and molecular formula of all photodegraded products are summarized in Table II. Extracted-ion chromatograms (EIC) of MF and its photodegraded products are shown in Fig. 3.

Table III. Apparent First-Order Rate Constants (k_{obs}) for the Photolysis of MF at pH 2.0–12.0

pH	$k_{obs}\times10^4$ $(min^{-1})^a\pm SD^b$	$t_{1/2}$ (min)	t_{90} (min)	Correlation coefficient
2.0	10.54±0.26	657	100	0.999
3.0	8.88±0.11	780	118	0.998
4.0	7.75±0.16	894	135	0.999
5.0	6.29±0.19	1,102	167	0.998
6.0	5.16±0.12	1,343	203	0.999
6.5	4.49±0.10	1,543	234	0.999
7.0	3.26±0.13	2,126	322	0.999
7.5	0.69±0.02	10,043	1,522	0.997
8.0	2.53±0.08	2,739	415	0.999
9.0	6.44±0.21	1,076	163	0.998
10.0	10.72±0.38	647	98	0.997
11.0	14.90±0.60	465	70	0.999
12.0	19.50±0.59	355	54	0.999

[a] The values of rate constants are relative and depend on specific experimental conditions including the light intensity
[b] $n=3$

The results of this study show that MF on UV irradiation in acid solution leads to the formation of five photodegraded products (MP-1 to MP-5) and in alkaline solution three photodegraded products (MP-1, MP-4, and MP-5). MP-1, MP-4, and MP-5 are common in both acid and alkaline solutions. The major end product in both the solutions is MP-4. Three of these products (MP-1, MP-2, and MP-3) have previously being reported on the photodegradation of MF in the solid state (19).

Kinetics of Photodegradation

The apparent first-order rate constants (k_{obs}) for the photodegradation of MF at pH 2.0–12.0 range from 0.69×10^{-4} (pH 7.5) to 19.50×10^{-4} min^{-1} (pH 12.0) (Table III), indicate that the rate of reaction increases with a decrease in pH in the acid region and increases with an increase in pH in the alkaline region. This increase in rate could result from

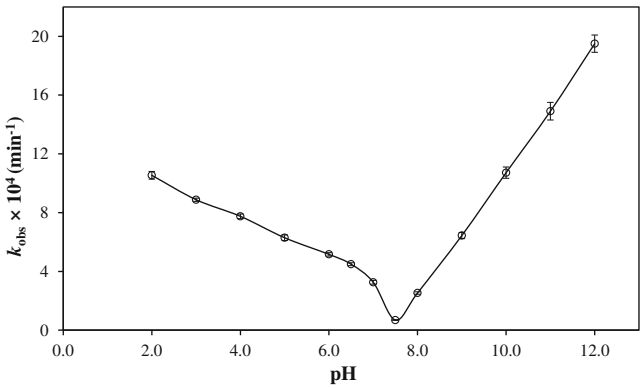


Fig. 4. k_{obs}–pH profile for the photodegradation of MF in aqueous solution

specific acid- and specific base-catalyzed degradation with maximum stability around pH 7.5. The apparent first-order rate constants for this reaction can be expressed by Eq. (1).

$$k_{obs} = k_0 + k_1[MF](H^+] + k_2[MF^+][H^+] + k_3[MF^{\pm}][H_2O] + k_4[MF][H_2O] + k_5[MF][OH^-] + k_6[MF^-][OH^-] \quad (1)$$

where k_0 is the rate constant for the uncatalyzed reaction, k_1 and k_2 are the second-order rate constants for specific acid catalysis of MF and MF^+, respectively, k_3 and k_4 are the first-order rate constants for the reactions of zwitterion $MF^{\pm}$, and neutral MF with water, and k_5 and k_6 are the second-order rate constants for specific base catalysis of MF and MF^-, respectively. The values of k_{obs} depend on the experimental conditions, including light intensity, and may be used for comparative purpose.

Effect of pH

The k_{obs}–pH profile for the photodegradation of MF is a V-shaped curve showing the behavior as indicated by the reactions represented by Eq. (1) and undergone by the cationic, zwitterionic, neutral, and anionic species of MF involving specific acid-base catalysis with a $pH_{minmax}=1/2$ ($pK_{a1}+pK_{a2}$)=7.5. It is evident from the k_{obs}–pH profile (Fig. 4) that the rate constant for the photodegradation of MF is minimum

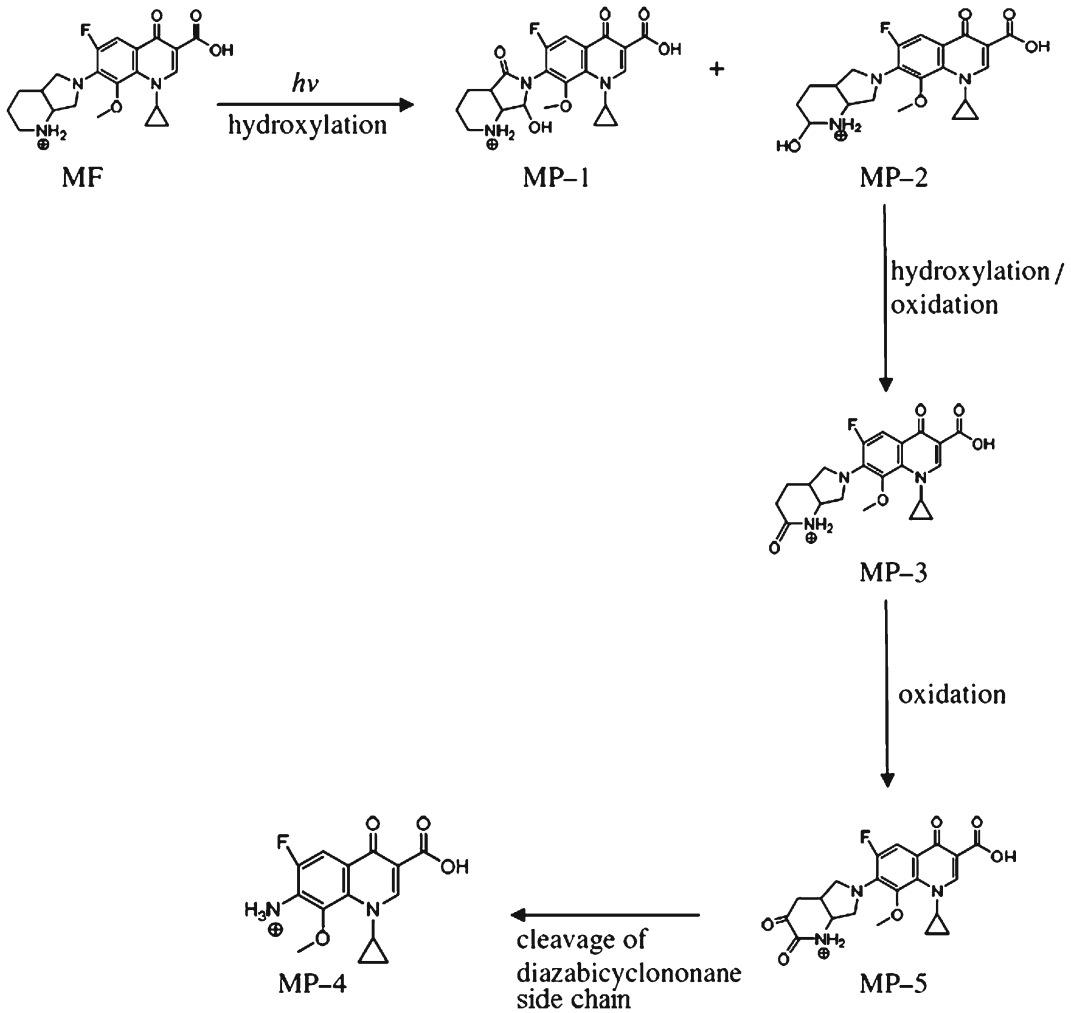


Fig. 5. Proposed pathway for the photodegradation of MF in acid solution

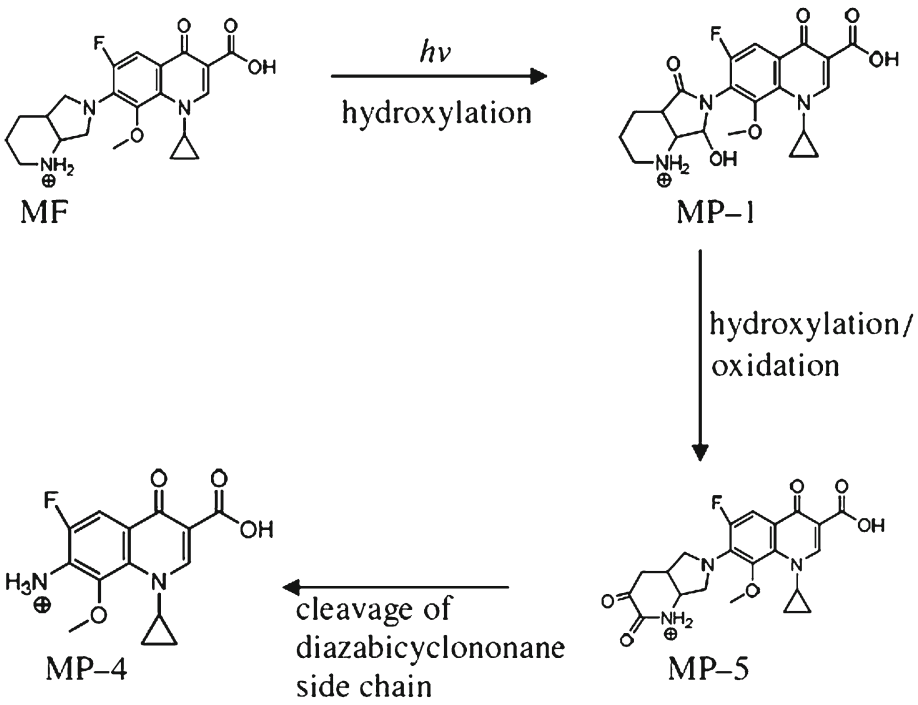


Fig. 6. Proposed pathway for the photodegradation of MF in alkaline solution

in the pH range 7–8, as indicated by the pK_a values for this drug. Since the photodegradation of MF is a specific acid-base catalyzed reaction, the second-order rate constants for the [H^+]- and [OH^-]-catalyzed reactions have been determined from the slopes of the plots of k_{obs} *versus* [H^+] and [OH^-] ion concentrations at different pH values as 6.61×10^{-2} and 19.20×10^{-2} M^{-1} min^{-1}, respectively. These rate constants indicate that the photodegradation of MF in alkaline solution is about three-fold faster than that of the acid solution. This could be due to the cleavage of the piperidine ring (pK_a 9.42) attached to pyrrole ring at C-7 of the molecule as a result of the higher reactivity of the MF excited triplet state. The V-shaped k_{obs}–pH profiles have been observed for the degradation of benzyl penicillin (40), ampicillin (41), carbenicillin (42), methicillin (43), chlordiazepoxide (44), hydrochlorothiazide (45), streptovitacin A (46), cocaine (47), cytarabine (48), nystatin (49), 5-azacytidine (50), and aztreonam (51). This indicates that MF undergoes a similar degradation behavior as shown by these drugs.

Table IV. Apparent First-Order Rate Constant (k_{obs}) for the Photodegradation of MF in Water and Organic Solvents

Solvents	Dielectric constant (25°C)	Inverse viscosity $(mPa\ s)^{-1}$	$k_{obs}\times10^4$ (min^{-1}) ±SD[a]
Water (pH 7.0)	78.5	1.000	3.26±0.13
Acetonitrile	38.5	2.898	2.04±0.10
Methanol	32.6	1.838	1.81±0.11
Ethanol	24.3	0.931	1.55±0.09
1-Propanol	20.1	0.514	1.35±0.05
1-Butanol	17.8	0.387	1.24±0.08

[a] $n=3$

Effect of Ionization

MF has two proton-binding sites (basic group in the 7-position and acid group in the 3-position) and exists in four species in aqueous solution, namely, cationic [MF^+], zwitterionic [$MF^{\pm}$], neutral [MF^0], and anionic [MF^-] forms at the molecular level. MF (pK_{a1} 5.69, pK_{a2} 9.42) undergoes several acid-base equilibria to form protonated and deprotonated species with a change in pH (52,53). This is evident from the loss of fluorescence of singly ionized species in acid and alkaline regions (54). The rate of photodegradation of MF in aqueous solution depends upon the reactivity of the ionic species existing in a particular pH range. The V-shaped k_{obs}–pH profile for the photodegradation of MF (Fig. 4) indicates the effect of ionization of the molecule on the rate of the reaction. The profile mainly represents the photodegradation of cationic and anionic species of MF in the acid and alkaline regions. It appears that both of these species are more susceptible to photodegradation compared to that of the zwitterionic and neutral species. An increase in protonated piperidine species in the acid range and anionic carboxylic species of the molecule in the alkaline range (^+H_2N–COO^-) appears to affects the rate of photodegradation. The photodegradation of MF is about 15-fold higher at pH 2.0 and 28-folds higher at pH 12.0 compared to that of pH 7.5 at which the molecule is most stable. The pH 7.5 may be considered useful for the formulation of liquid preparations of MF.

Mode of Photodegradation

MF is known to undergo several acid-base equilibria in aqueous solution leading to the formation of ionic species in different pH regions (17,52,53). These ionic species participate in the photodegradation of MF and the rate of reaction would

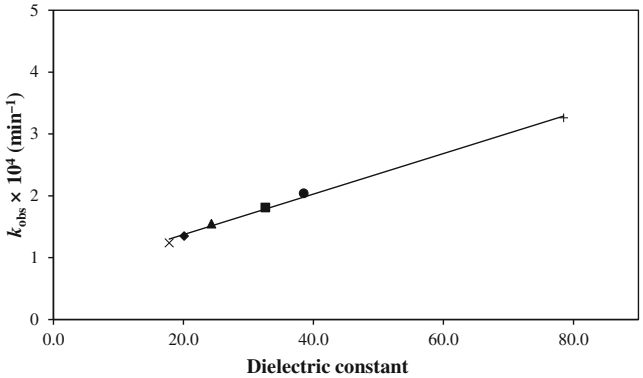


Fig. 7. A plot of k_{obs} *versus* dielectric constant for the photodegradation of MF in various solvents. (*plus sign*) water; (*black) acetonitrile; (black square) methanol; (black triangle) ethanol; (black diamond) 1-propanol; (multiplication sign) 1-butanol*

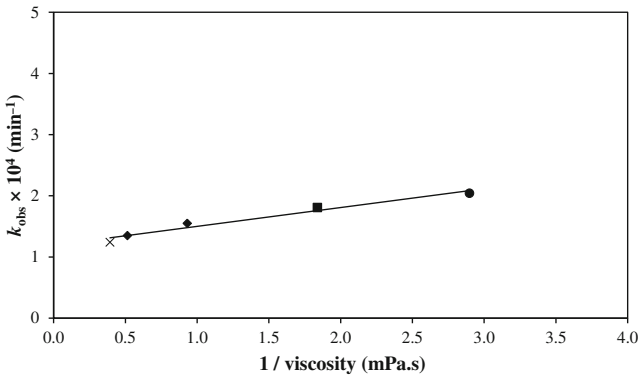


Fig. 8. A plot of k_{obs} *versus* inverse of viscosity for the photodegradation of MF in various solvents. (*plus sign*) water; (*black) acetonitrile; (black square) methanol; (black triangle) ethanol; (black diamond) 1-propanol; (multiplication sign) 1-butanol*

depend on the susceptibility of a particular species to UV excitation and degradation. Different pathways have been suggested for the photodegradation of MF and the formation of degradation products identified by NMR and MS techniques (18,19,54). In view of the greater susceptibility of MF to photodegradation in alkaline solution, compared to that of the acid solution, an increase in rate with pH above seven could be due to the cleavage of the piperidine ring and further reactions to form different degradation products.

The photodegradation of MF involves several pathways for the formation of primary and secondary photodegradation products (18), including: (a) unimolecular photoreactions proceeding *via* the excited triplet states and (b) bimolecular reactions where the excited state of the drug molecule attacks another drug molecule causing electron or hydrogen transfer from the electron-rich moiety present. This is a general process leading to N–N bond cleavage and is more important with tertiary amines and with five membered rings. Thus, photodegradation may occur in MF bearing a 2,8-diazabicyclo[4.1.0] nonane side chain. The secondary processes involve the degradation of the quinoline ring (12,21,22). The MF photodegraded products obtained by HPLC–FD and UPLC–MS/MS analysis have been reported (18,19).

In the present case, five photodegraded products (MP-1 to MP-5) of MF have been identified by LC–MS/MS analysis. In the photodegradation sequence other minor products may also be present. In view of the photodegraded products identified, schemes for the proposed pathways of photodegradation of MF in acid and alkaline solutions are presented.

Acid Solution

MF on UV excitation undergoes hydroxylation of the piperidine ring (MP-2) and hydroxylation and photooxidation of the pyrrole ring in the diazabicyclononane side chain (MP-1). This is followed by stepwise oxidation of the piperidine ring in the molecule (MP-3, MP-5). MP-5 may undergo further reaction by the cleavage of the diazabicyclononane side chain to produce the quinolone derivative (MP-4) as the final product (Fig. 5). The rate and extent of formation of these products would depend on the pH and acid-base equilibria in the region.

Alkaline Solution

MF on light absorption undergoes hydroxylation and photooxidation of the pyrrole ring (MP-1) and oxidation of piperidine ring (MP-5) in the side chain. This is followed by cleavage of the diazabicyclononane side chain of MP-5 to form the quinolone derivative (MP-4) (Fig. 6), as in the case of the acid solution. However, the detection of only three products in alkaline solution indicates that the reaction is faster in the alkaline solution compared to that of the acid solution. This could be due to the ability of any intermediates involved in the process to form the detected products. The mode of photodegradation of MF is similar in acid and alkaline media as a result of the specific acid-base catalysis in the whole pH range.

Effect of Solvent

MF possesses a quinolone nucleus with nitrogen atom in position 1, a carboxylic group at C-3, a keto group at C-4 and a diazabicyclononyl moiety at C-7. The presence of both the carboxylic and amino groups impart an acid-base character to the molecule which is influenced by the physicochemical properties of the solvent (55) and hence the reaction undergone by MF. The photodegradation of MF in organic solvents has been studied and the values of k_{obs} for these reactions are reported in Table IV. A plot of k_{obs} *versus* solvent dielectric constant is linear (R^2=0.998) (Fig. 7), indicating that the reaction involves a polar intermediate in the degradation pathway as observed in the case of photolysis of flavins (56,57), fluoroquinolones (12,21,22) and levofloxacin (28) in organic solvents. An increase in k_{obs} with solvent dielectric constant suggests a greater degree of solvent interaction to promote the reaction. It has also been observed in the present study that there is a linear relation between k_{obs} and the inverse of viscosity of the medium (R^2=0.998) (Fig. 8). The solvent viscosity may influence the reactivity of the excited triplet state of MF as a consequence of the underlying diffusion controlled processes. A similar behavior of the effect of solvent viscosity on the photodegradation of flavins (28,56) and levofloxacin has been observed (28). The results of this study emphasize the importance of solvent characteristics that may influence the rate of photodegradation of a drug substance. In the case of MF, the photodegradation of the molecule is

promoted in a polar medium and is inhibited by the viscosity of the solvent. It has been observed that the rate of photodegradation of MF is faster in aqueous solution compared to that of the organic solvents due to a change in the mode of reaction.

CONCLUSION

The photodegradation of MF in aqueous solution (pH 2.0–12.0) leads to the formation of several degradation products. The reaction involves specific acid-base catalysis and is represented by a V-shaped k_{obs}–pH profile. MF undergoes several acid-base equilibria in aqueous solution and its photodegradation depend on the reactivity of the particular ionic species (cationic, zwitterionic, and anionic) present in the pH range. The rate of MF photodegradation is faster in the alkaline solution compared to that of the acid solution. This may be due to greater susceptibility of the excited triplet state of MF to undergo degradation in the alkaline solution. Pathways have been proposed for the photodegradation of MF in acid and alkaline media in the light of the products identified. MF exhibits maximum stability in the pH range 7–8 which is suitable for maintaining the pH of its liquid preparations. The photodegradation of MF in organic solvents depends on solvent characteristics and the rate constants for degradation are a linear function of solvent dielectric constants and an inverse function of solvent viscosity.

REFERENCES

1. Sweetman SC. The complete drug reference. 36th ed. London: Pharmaceutical Press; 2009. p. 302.
2. British Pharmacopoeia. Monograph on moxifloxacin. London: Her Majesty's Stationary Office; 2013. Electronic version.
3. O'Neil MJ. The Merck Index. 15th ed. Cambridge: The Royal Society of Chemistry; 2013. p. 1171.
4. Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspective. Int J Antimicrob Agents. 2000;16:5–15.
5. Eliopulos GM. Activity of newer fluoroquinolones in vitro against gram-positive bacteria. Drugs. 1999;58:23–8.
6. Sharma SK, George N, Kdhiravan T, Saha PK, Mishra HK, Hanif M. Prevalence of extensively drug resistant tuberculosis among patients with multidrug-resistant tuberculosis: a retrospective hospital-based study. Indian J Med Res. 2009;130:392–5.
7. Soni K. Fluoroquinolones: chemistry & action—a review. Indo Glob J Pharm Sci. 2012;2:43–53.
8. Maruri F, Sterling TR, Kaiga AW, Blackman A, Vander Heigden YF, Mayer C, *et al*. A systematic review of gyrase mutations associated with fluoroquinolone-resistant *Mycobacterium tuberculosis* and a propose gyrase numbering system. J Antimicrob Chemother. 2012;67:819–31.
9. Tarkase KN, Admane SS, Sonkhede NG, Shejwal SR. Development and validation of UV spectrophotometric methods for determination of moxifloxacin HCl in bulk and pharmaceutical formulations. Der Pharma Chemica. 2012;4:1180–5.
10. De Guidi G, Giuffrida S, Monti S, Pisu PS, Sortino S, Costanzo LL. Molecular mechanism of phtosensitization induced by drugs XIV: two different behaviors in the photochemistry and photosensitization of antibacterials containing a fluoroquinolone like chromophore. Int J Photoenergy. 1999;1:1–6.
11. Viola G, Facciola L, Canton M, Vedaldi D, Acqua FD, Aloisi GG, *et al*. Photophysical and phototoxic properties of the antibacterial fluoroquinolones levofloxacin and moxifloxacin. Chem Biodivers. 2004;1:782–800.
12. Hidalgo ME, Pessoa C, Fernandez E, Cardenas AM. Comparative determination 232 of photodegradation kinetics of quinolones. J Photochem Photobiol A Chem. 1993;73:135–8.
13. Burhenne J, Ludwig M, Spiteller M. Polar photodegradation products of quinolones determined by HPLC/MS/MS. Chemosphere. 1999;38:1279–86.
14. Kummerer K. Antibiotics in the aquatic environment—a review—part I. Chemosphere. 2009;75:417–34.
15. United States Pharmacopoeia 30–National Formulary 25. Rockville, MD, USA: United States Pharmacopoeial Convention, Inc., Electronic version; 2007.
16. Lorenzo F, Navaratnam S, Edge R, Allen NS. Primary photophysical properties of moxifloxacin—a fluoroquinolone antibiotic. Photochem Photobiol. 2008;84:1118–25.
17. Doorslaer XV, Demeestere K, Heynderickx PM, Langenhove HV, Dewulf J. UV-A and UV-C induced photolytic and photocatalytic degradation of aqueous ciprofloxacin and moxifloxacin: reaction kinetics and role of adsorption. Appl Catal B Environ. 2011;101:540–7.
18. Sturini M, Speltini A, Maraschi F, Profumo A, Pretali L, Irastorza EA, *et al*. Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight. Appl Catal B Environ. 2012;119–120:32–9.
19. Hubicka U, Zmudzki P, Talik P, Zuromska BW, Krzek J. Photodegradation assessment of ciprofloxacin, moxifloxacin, norfloxacin and ofloxacin in the presence of excipients from tablets by UPLC-MS/MS and DSC. J Chem Cent. 2013;7:1–12.
20. Torniainen K, Tammilehto S, Ulvi V. The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin. Int J Pharm. 1996;132:53–61.
21. Burhenne J, Ludwig M, Nikoloudis P, Spiteller M. Photolytic degradation of fluoroquinolone carboylic acid in aqueous solution. Part I: primary photoproducts and halflives. Environ Sci Pollut Res. 1997;4:10–5.
22. Burhenne J, Ludwig M, Spiteller M. Photolytic degradation of fluoroquinolone carboylic acid in aqueous solution. Part II: isolation and structural elucidation of polar photometabolities. Environ Sci Pollut Res. 1997;4:61–7.
23. Lovdahl MJ, Priebe SR. Characterization of clinafloxacin photodegradation products by LC-MS/MS andNMR. J Pharm Biomed Anal. 2000;23:521–34.
24. Salgado HRN, Moreno PRH, Braga AL, Schapoval EES. Photodegradation of sparfloxacin 259 and isolation of its degradation products by preparative HPLC. Rev Cienc Farm Basica Apl. 2005;26:47–54.
25. Motwani SK, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar S, *et al*. Stability indicating high performance thin-layer chromatographic determination of gatifloxacin as bulk drug and form polymeric nanoparticles. Anal Chim Acta. 2006;576:253–60.
26. Budai M, Grof P, Zimmer A, Papai K, Klebovich I, Ludanyi K. UV light induced photodegradation of liposomes encapsulated fluoroquinolones. J Photochem Photobiol A Chem. 2008;198:268–73.
27. Wang J, Li W, Li C-G, Hu Y-Z. Photodegradation of fleroxacin injection: different products with different concentration levels. AAPS PharmSciTech. 2011;12:872–8.
28. Ahmad I, Bano R, Sheraz MA, Ahmed S, Mirza T, Ansari SA. Photodegradation of levofloxacin in aqueous and organic solvents: a kinetic study. Acta Pharma. 2013;63:221–7.
29. Hutchinson DJ, Johnson CE, Klein KC. Stability of extemporaneously prepared moxifloxacin oral suspensions. Am J Health Syst Pharm. 2009;66:665–7.
30. Kumar MT, Srikanth G, Rao JV, Rao KS. Development and validation of HPLC-UV method for the estimation of levofloxacin in human plasma. Int J Pharm Pharm Sci. 2011;3:247–50.
31. Rama Subbaiah P, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM. Method development and validation for estimation of moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta. 2010;1:1–2.
32. Sultana N, Arayne MS, Akhtar M, Shamim S. High-performance liquid chromatography assay for moxifloxacin in bulk, pharmaceutical formulations and serum: application to in-vitro metal interactions. J Chin Chem Soc. 2010;57:1–10.

33. Dewani AP, Barik BB, Kanungo SK, Wattyani BR, Chandewar AV. Development and validation of RP-HPLC method for the determination of moxifloxacin in presence of its degradation products. Am-Eurasian J Sci Res. 2011;6:192–200.
34. Kunagu VS, Janardhan M. Development and validation of stability-indicating RP-HPLC method for estimation of moxifloxacin in moxifloxacin HCl tablets. Int J Pharm Invent. 2012;2:24–33.
35. Wang N, Zhu L, Zhao X, Yang W, Sun H. Improved HPLC method for the determination 285 of moxifloxacin in application to a pharmacokinetics study in patients with infectious diseases. ISRN Pharmacol. 2013;2013:1–7.
36. Motwani SK, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar S. Application of a validated stability indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin. Anal Chim Acta. 2007;582:75–82.
37. Devi ML, Chandrasekhar KB. A validated, specific stability-indicating RP-LC method for moxifloxacin and its related substances. Chromatographia. 2009;69:993–9.
38. Hatchard CG, Parker CA. A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. Proc R Soc Lond A. 1956;235:518–36.
39. Ahmed S, Sheraz MA, Yorucu C, Rehman IU. Quantitative determination of tolfenamic acid and its pharmaceutical formulation using FTIR and UV spectrometry. Cent Eur J Chem. 2013;11:1533–41.
40. Finholt P, Jurgensen G, Kristiansen H. Catalytic effect of buffers on degradation of penicillin G in aqueous solution. J Pharm Sci. 1965;54:387–93.
41. Connors KA, Amidon CL, Stella VJ, editors. Chemical stability of pharmaceuticals: a hand book for pharmacists. 2nd ed. New York: Wiley; 1986. p. 198–207. 250–256.
42. Zia H, Teharan M, Zargarbashi R. Kinetics of carbenicillin degradation in aqueous solution. Can J Pharm Sci. 1974;9:112–7.
43. Schwartz MA, Bara E, Rubycz I, Granatek AP. Stability of methacillin. J Pharm Sci. 1965;54:149–50.
44. Maulding HV, Nazareno JP, Pearson JE, Michaelis AF. Practical kinetics III: benzodiazepine hydrolysis. J Pharm Sci. 1975;64:278–84.
45. Mollica JA, Rehm CR, Smith JB, Govan HK. Hydrolysis of benzothiadiazines. J Pharm Sci. 1971;57:1380–4.
46. Notari RE, Caiola SM. Catalysis of streptovitacin A dehydration: kinetics and mechanism. J Pharm Sci. 1969;58:1203–8.
47. Garrett ER, Seyda K. Prediction of stability in pharmaceutical preparations XX: stability evaluation and bioanalysis of cocaine and benzoylecgonine by high performance liquid chromatography. J Pharm Sci. 1983;72:258–71.
48. Notari RE, Chin ML, Wittebort R. Arabinosylcytosine stability in aqueous solution: 313 pH profile and shelf life productions. J Pharm Sci. 1972;61:1189–96.
49. Hamilton-Miller JMT. The effect of pH and of temperature on the stability and bioactivity of nystatin and amphotericin B. J Pharm Pharmacol. 1973;25:401–7.
50. Notari RE, DeYoung JL. Kinetics and mechanisms of degradation of the antileukemic agent 5-azacytidine in aqueous solution. J Pharm Sci. 1975;64:1148–57.
51. Langlois M-H, Montagut M, Dubost J-P, Grellet J, Saux M-C. Protonation equilibrium and lipophilicity of moxifloxacin. J Pharm Biomed Anal. 2005;37:389–93.
52. Lemaire S, Tulkens PM, Bambeke FV. Contrasting effects of acidic pH on the extracellular and intracellular activities of the anti-gram-positive flouroquinolones moxifloxacin and delafloxacin against *Staphylococcus aureus*. Antimicrob Agents Chemother. 2011;55:649–58.
53. Nangia A, Lam F, Hung CT. A stability of aqueous solution of norfloxacin. Drug Dev Ind Pharm. 1991;17:681–94.
54. Hubicka U, Krzek J, Zuromska B, Walczak M, Zylewski M, Pawlowski D. Determination of photostability and photodegradation products of moxifloxacin in the presence of metal ions in solutions and solid phase. Kinetics and identification of photoproducts. Photochem Photobiol Sci. 2012;11:351–7.
55. Park HR, Kim TH, Bark KM. Physicochemical properties of quinolone antibiotics in various environments. Eur J Med Chem. 2002;37:443–60.
56. Ahmad I, Tollin G. Solvent effects on flavin electron transfer reactions. Biochemistry. 1981;20:5925–8.
57. Ahmad I, Fasihullah Q, Vaid FHM. Photolysis of formylmethylflavin in aqueous and organic solvents. Photochem Photobiol Sci. 2006;5:680–5.