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KINETIC AND THERMODYNAMIC STUDIES OF MOXIFLOXACIN HYDROLYSIS IN THE PRESENCE AND ABSENCE OF METAL IONS IN ACIDIC SOLUTIONS

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Abstract: Chromatographic and densitometric method for determination of moxifloxacin in the presence of products of acidic hydrolysis was developed. The established method had suitable specificity, precision, good accuracy and high sensitivity.In addition, stability of moxifloxacin in acidic solutions at temperature 90˚C and 110˚C in the presence and absence of metal ions, such as Cu(II), Fe(III), Zn(II), and Al(III) was studied. It was proved that decomposition of moxifloxacin proceeds according to kinetics of the first-order reaction and is dependent on temperature, incubation time and the type of the metal ion. Based on the calculated kinetic (k, $t_{0.1}$ and $t_{0.5}$) and thermodynamic (E_a) parameters, it was observed that among studied ions the highest effect on decomposition process of moxifloxacin had Cu(II) ions. The liquid chromatography coupled with mass spectrometry detection (LC-MS) and proton nuclear magnetic resonance (^{1}H NMR) techniques have been used to identify degradation products of moxifloxacin.

Keywords: fluoroquinolones, ^{1}H NMR, LC-MS, metal ions, moxifloxacin, stability

Moxifloxacin (1-cyclopropyl-7-[(1*S*, 6*S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline-3-carboxylic acid, monohydrochloride) is a fourth generation synthetic fluoroquinolone chemotherapeutic agent (1). It possesses excellent activities against a variety of different types of Gram-negative (*Streptococcus pneumoniae* and *Staphylococcus aureus*), Gram-positive (*Enterobacteriaceae* family, and *Pseudomonas aeruginosa*) and anaerobic bacteria (*Bacteroides* and *Clostridium*). Its activity against atypical bacteria of *Chlamydia spp*., *Mycoplasma spp.,* and *Legionella spp*. genera and bacilli of genus *Mycobacterium* is of high value (2–5).

The mechanism of action of moxifloxacin, similar to other fluoroquinolones, lies in inhibiting the activity of two bacterial enzymes – DNA gyrase (topoisomerase II) and topoisomerase IV, which regulate spatial arrangement of DNA in bacterial cells. These proteins can cut both strands of the nucleic acid and rejoin them. Inhibiting the activity of those enzymes by formation of irreversible complex drug/enzyme/DNA, disables DNA synthesis and leads to the bacterial cell death (3, 4).

According to the available literature, high pressure liquid chromatography (HPLC) technique is mainly used for analysis of moxifloxacin in pharmaceutical preparations and biological material (5–14). This technique was used for identification of moxifloxacin and its decomposition products (1, 15, 16). The ligand-exchange liquid chromatography with the use of a chiral modifier in stationary phase was used for separation of moxifloxacin and its (R, R)-isomer (17).

The LC-MS/MS technique was used for identification and quantification of moxifloxacin in human plasma and cerebrospinal fluid (18, 19).

The TLC method for identification of lomefloxacin, moxifloxacin and sparfloxacin and their decomposition products was recommended by Salem et al. (20) Identification of moxifloxacin was performed on TLC plates covered with silica gel with the mobile phase: 0.3 M ammonium acetate solution – conc. ammonia – n-propanol (1: 1: 8, v/v/v). The spots on chromatograms were recorded densitomerically at λ = 290 nm (20).

For stress studies with moxifloxacin, Motwani et al. used the silica gel coated HPTLC plates and

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the mobile phase: propanol – ethanol – 6 M ammonia (4 : 1 : 2, v/v/v); the spots were densitometrically recorded at λ = 298 nm (21).

Capillary electrophoresis with florescence detection induced with laser for identification of moxifloxacin in body fluids and capillary electrophoresis with spectrophotometric detection in the UV range for studies of enantiomeric purity (22, 23) should be mentioned among other separation methods. Besides separation methods, differential pulse polarography (24), differential pulse voltammetry (25) and spectrophotometry (20, 26) or spectrofluorimetry (27, 28) are applied for identification of moxifloxacin.

There are not so many available papers on studies of acidic hydrolysis of moxifloxacin. Two publications report the acidic hydrolysis carried out using 0.5 M HCl at 50°C for 5 days; no presence of decomposition products was observed (15). Djurdjevic et al., demonstrated around 0.3% decomposition and they detected two decomposition products in 0.1 M HCl solution after 3 h heating at 50°C (16).

After 10 h heating in 2 M HCl at 100°C, Salem et al. demonstrated the presence of one decomposition product that was identified by infrared (IR) spectroscopy as a product of moxifloxacin decarboxylation (20).

During stress studies with moxifloxacin, Motwani et al. observed significant decomposition of the compound after 3 h of heating at 40 to 90°C in 1 M HCl; however, they did not identified decomposition products (21).

Developent of chromatographic-densitometric method for determination of stability of moxifloxacin in acidic solutions and the influence of chosen Cu(II), Fe(III), Zn(II) and Al(III) ions on kinetic (k, $t_{0.1}$, $t_{0.5}$) and thermodynamic (E_a) parameters was the aim of this study. In addition, LC-MS and ^{1}H NMR was used to determine the structure of hydrolysis products.

EXPERIMENTAL

Chemicals and reagents

All reagents used for studies had analytical quality and were manufactured by POCH Gliwice, Poland or Merck, Darmstadt, Germany.

Standard substance

Moxifloxacin hydrochloride series Strasbourg Cedex; Code:Y0000703; Id: 002IC0; Council of Europe – EDQM CS; Cat No. T30026 F-6081.

Apparatus

Densitometer –TLC Scanner 3 with WinCats 1.3.4 software (Camag, Muttenz, Switzerland). Sample applicator – Linomat V Camag (Muttenz, Switzerland). TLC plates – 10 × 12 cm (cut from 20 × 20 cm precoated TLC sheets of silica gel 60 F_{254} on aluminium; Art. 1.05548; Merck Darmstadt, Germany). Chromatographic chamber – 17.5 × 16 × 8.2 cm in size (Sigma – Aldrich, Cat. No. Z20, 415-3). LC system – Agilent 1100 (Agilent Technologies, Waldbronn, Germany) LC system. Column – XBridge C18 (30 mm × 2.1 mm, 3.5 mm, Waters, Ireland). Mass spectrometer – Applied Biosystem MDS Sciex (Concord, Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. NMR spectrophotometer – Mercury VX 300 MHz; Varian (USA).

Solutions

Metal salts solutions

To prepare the solutions of metal ions at 0.1 mol/L concentration the following salts were used: (1.2488 g) $CuSO_4 \cdot 5H_2O$, (1.4337 g) $ZnSO_4 \cdot 7H_2O$, (0.9988 g) $Fe_2(SO_4)_3 \cdot H_2O$, (1.6753 g) $Al_2(SO_4)_3 \cdot 18H_2O$. Above given weighed portions of salts were transferred to 50 mL flasks and filled with water to required volume. Directly for testing, the obtained solutions were diluted with the same solvent to a final concentration of 0.025 mol/L.

Standard solution

The amount of 0.2 g of moxifloxacin, weighted with a precision of 0.1 mg, was dissolved in the volume of 50 mL of methanol, and filled up to 100 mL with the same solvent. For method validation, a solution with concentration of 60 µg/mL was prepared, and later appropriately diluted.

Preparation of samples

The amount of 3.0 mL 0.2% methanol solution of moxifloxacin, 3.0 mL HCl (3 mol/L) solution and 1.0 mL of distilled water or solution with proper salt with 0.025 mol/L concentration of metal ion was added to 8.0 mL vials. Final concentration of HCl in the studied samples was 1.3 mol/L. The vials were tightly closed with plastic cap and placed in incubator at temperatures 90°C and 110°C to prevent them from direct light. The amount of 0.5 mL of solution diluted with methanol to the final volume of 10.0 ml was collected after 24, 48 and 72 h for further analysis. In case of sediment occurrence, the solutions were centrifuged at 3000 × g for 10 min and the supernatant was analyzed.

TLC analysis

A chromatographic-densitometric method was used for identification of moxifloxacin and its hydrolysis products. The amounts of 10 mL of solutions were applied by Linomat V in a form of 8 mm bands on TLC plates of 10 × 12 cm in size. The chromatograms were developed to the height of 11.5 cm using mobile phase: methylene chloride – ethanol – toluene – n-butanol – ammonia 25% – water (6:6:2:3:1.8:0.3, v/v/v/v/v), and then dried at room temperature for 30 min. The peak areas were scanned and recorded at λ 294 nm. The absorption spectra within the range from 200 nm to 400 nm were recorded for identification purposes. Identification of constituents was based on comparison of retardation factor (R_f) values and the absorption spectra. The percentage share of the constituent (%i) was calculated from the ratio of peak area of evaluated constituent (Ai) to the sum of all peak areas (ΣA) on densitogram according to the formula %i = (Ai/SA) × 100.

The study results represent average values obtained from two measurements.

The method was validated for specificity, linearity, precision, recovery, limit of detection and limit of quantitation according to ICH guideline (28). The specificity of the method is its ability to measure analyte response in the presence of potential impurities. In order to determine the specificity, 20 µL of moxifloxacin solutions without and with ions of studied metals, after hydrolysis were spotted onto TLC plates. For linearity studies aliquots of 0.03 µg per band to 2.5 µg per band of standard solutions were applied on a TLC plate. Further analytical procedure was as described above. Determination of linearity was made in three replicates. Linearity was assessed as a relationship between mean peak area and concentration from 0.06 to 1.5 µg per band and was reported as the linear calibration equations and the correlation coefficients (r). For precision, repeatability of sample application and determination of peak area were assessed by making 5 replicates of different amounts of standard solution (0.12, 0.6, 1.2 µg per band). Assay of accuracy was determined by estimating recovery percentage for moxifloxacin. Known amounts of standard substance of moxifloxacin (80%, 100%, 120%) were added to model solution of this compound. Recovery in percentage value was calculated on the basis of determined content of moxifloxacin to weighed amount. Each level was analyzed in triplicate and a mean value from 9 analyses was taken as a result. In order to asses limit of detection (LOD) and limit of quantitation (LOQ) decreasing volumes (5, 4, 3, 2, 1 µg per band) of standard solution of the concentration 6 µg/mL were applied on a TLC sheet. Further analytical procedure was as described above. Peak area was measured and signal to noise ratio 3:1 was regarded as LOD, whereas signal to noise ratio 10:1 was regarded as LOQ.

LC/MS/MS analysis

High-performance liquid chromatography conditions

Liquid chromatography was performed using an Agilent 1100 HPLC system consisting of degasser, a gradient pump, an autosampler and a DAD detector. Chromatographic separation was carried out with a commercially available XBridge C18 column set at 30°C.

The mobile phase consisted of acetonitrile and water with an addition of 0.01% formic acid was set at a flow rate of 0.6 mL/min using a gradient elution. Two solvent mixtures were used. Solvent A: acetonitrile – formic acid (0.01%) and solvent B: water – formic acid (0.01%). The following gradient was used: 0–5 min, 0–100% A; 5–7 min, 100% A; 7–8 min, 100–0% A; 8–15 min, 100% B. A sample volume of 20 µL was injected onto the analytical column for compound analysis.

Mass spectrometry conditions

Mass spectrometric detection was performed on an Applied Biosystems MDS Sciex API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface and performed in the positive ion mode. The mass scanning was done on quadrupole Q1 in full scan mass spectrum using the mass range from m/z 50 to 1000 amu.

A standard solution of polypropylene glycol was used for instrument tuning and mass calibration according to the Applied Biosystems manual. The mass spectrometer was operated with a dwell time of 200 ms, and a 5 ms delay between scans for each transition in full scan mass spectrum on quadrupole Q1.

To find the optimal parameters of the ion source and the ion path, the mass spectrometer was tuned by direct infusion of studied drug at a concentration of 10 ng/mL using a Hamilton syringe pump set at 5 µL/min.

The ion source parameters were as follows: ion spray voltage (IS): 5500 V; nebulizer gas (gas 1): 40 psi; turbo gas (gas 2): 45 psi; temperature of the heated nebulizer (TEM): 300°C, and curtain gas (CUR): 10 psi. Nitrogen (99.9%) from Peak NM20ZA was used as the curtain and collision gas.

Table 1. The kinetic results of moxifloxacin decomposition in the presence and absence of metal ions at temperature 90°C and 110°C.

Temperature [°C]	Ion	Rate constant $k \times 10^{-2}$ [h^{-1}]	$t_{0.1}$ [h]	$t_{0.5}$ [h]	Correlation coefficient
90	Cu(II)	0.74	14.23	93.65	0.9312
	Fe(III)	0.19	55.42	364.74	0.9769
	Al(III)	0.18	59.76	393.30	0.9478
	Zn(II)	0.18	59.93	394.42	0.9645
	None	0.16	65.81	433.13	0.9777
110	Cu(II)	3.27	3.22	21.19	0.9998
	Fe(III)	2.16	4.88	32.08	0.9994
	Al(III)	2.10	5.01	33.00	0.9981
	Zn(II)	1.98	5.32	35.00	0.9831
	None	1.54	6.84	45.00	0.9998

The ion path parameters for moxifloxacin were as follows: declustering potential (DP): 10 V; focusing potential (FP): 350 V; entrance potential (EP): 10 V, electron multiplier (CEM): 2850 V. Data acquisition and processing were accomplished using the Applied Biosystems Analyst version 1.4.2 software.

^{1}H NMR analysis

Preparing samples for ^{1}H NMR analysis

To identify the degradation products by ^{1}H NMR it was necessary to isolate individual constituents from the chromatograms. For this purpose, having developed chromatograms and after location of bands of degradation products, appropriate layers of gel were scraped off. The silica gel was shaken mechanically with 5.0 mL of methanol for 1 h, and then filtered through a C_8 column (Bakerbond spe™, J.T. Baker, Deventer, Holland). The solvent was removed by distillation until a dry mass was obtained.

^{1}H NMR conditions

NMR spectra were measured in a mixture of $CDCl_3$ and CD_3OD on a Varian Mercury-VX 300 spectrometer operating at 300.08 MHz (^{1}H) and 282.32 MHz (^{19}F), chemical shifts (δ in ppm) were referenced to solvent lock signal. All spectra were acquired at ambient temperature. Due to low sample concentration, for each ^{1}H NMR spectra 256 scans were accumulated with spectral width of 4.2 kHz and 16 k data points. ^{19}F spectrum was recorded with spectral width of 56 kHz and 64 k data points, 512 scans were accumulated for ^{19}F spectrum acquisition.

RESULTS AND DISCUSSION

TLC analysis

Well-developed peaks and good separation of moxifloxacin from hydrolyzed products were obtained in the developed chromatographic conditions. Retardation factors were: R_f 0.32 for moxifloxacin and 0.15, 0.29 and 0.89 for degradation products. Mobile phase: methylene chloride – ethanol – toluene – n-butanol – ammonia 25% – water (6 : 6 : 2 : 3 : 1.8 : 0.3, v/v/v/v/v) used in these studies for determination of moxifloxacin differs from those described in the literature (20, 21). The method was validated according to ICH requirements (29).

Under the established method conditions, no mutual peak interference, or influence of other constituents present in the samples, was observed. Therefore, it is considered that the developed method meets the specificity criteria. The method is characterized by a wide linear range, from 0.06 µg per band to 1.5 µg per band, with good correlation ($r = 0.9900$). The regression coefficient is $P = 9354.5c + 2766.4$, and the standard estimation error equal $S_e = 808.2$. This method has high sensitivity with LOD = 12 ng per band and LOQ = 30 ng per band. Good precision and intermediate precision RSD does not exceed 1.08% and the recovery from 100.34% to 101.50%.

Acidic hydrolysis of moxifloxacin

Durability studies of moxifloxacin in acidic environment have been an element of many publications in which authors demonstrated the presence of

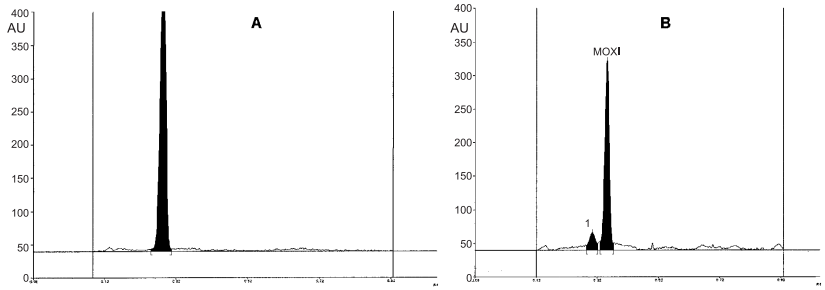

Figure 1. The densitogram of moxifloxacin solution in hydrochloric acid in the absence of metal ions: **A** – before heating, **B** – after heating at 90°C for 72 h. Peak 1 – the degradation product

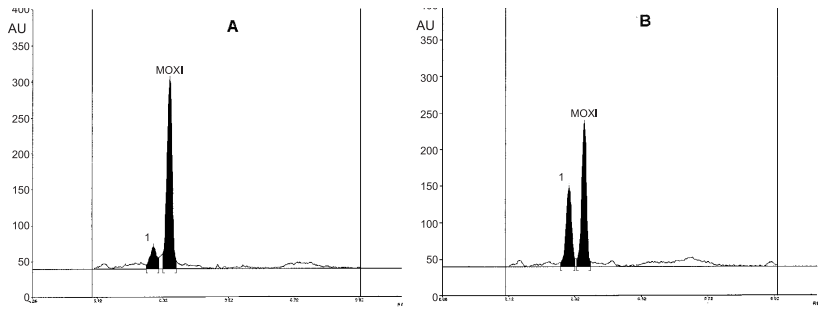

Figure 2. Densitograms of moxifloxacin hydrolysis in the presence of metal ions: Fe(III) (**A**), Cu(II) (**B**) after 72 h heating at 90°C. Peak 1 – degradation product P-2

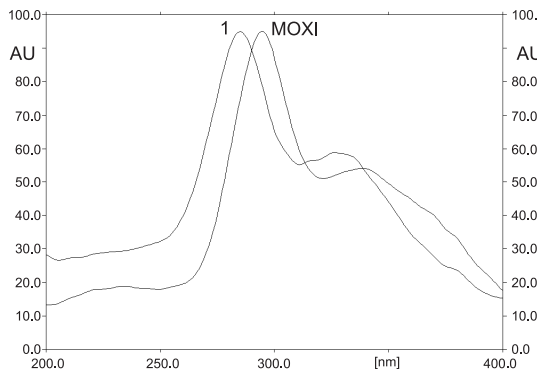

Figure 3. Absorption spectra of moxifloxacin (MOXI) and its degradation product P-2 (**1**) after incubation for 72 h at 90°C

one decomposition product obtained in the process of decarboxylation (20) or few decomposition products which structure has not been identified yet (16, 21).

In the searched literature, there was lack of data concerning durability of moxifloxacin in the presence of metal ions, which may result in some type of interaction during compound application in medical care. Acidic hydrolysis of fluoroquinolones with the presence of metal ions has been subjected of many studies; however, these studies only concerned ciprofloxacin and norfloxacin (30). Moxifloxacin differs from the above-mentioned fluoroquinolones at positions 7 and 8, and this difference has an influence on physicochemical properties and durability of the compound (31–33).

During selection of metal ions for studies, we based our decision on the presence of metal ions in many, commonly used diet supplements (Cu(II), Fe(III), Zn(II)) or alimentary tract coating agents (Al(III)), which can be administered during therapy with moxifloxacin.

In preliminary studies, no change was observed in moxifloxacin solutions in the presence and absence of metal ions before incubation at 90°C and 110°C was applied. There was only one peak representing studied compound with R_f 0.32 value on recorded densitograms (Fig. 1A).

Decomposition of moxifloxacin in the presence and absence of ions proceeds in solutions not earlier than after 24 h of heating at 90°C. This decomposition intensifies with the increase of heating time. After 72 h of heating, the decomposition process of moxifloxacin solutions in the absence of metal ions did not exceed 10%. On the other hand, the decomposition process was faster in the presence of metal ions: Cu(II) 41.92%, Fe(III) 13.61%, Zn(II) 12.8% and Al(III) 11.25%.

Besides moxifloxacin peak with R_f 0.32 value, only one extra peak (P-2) with R_f 0.29 value occurred on all the recorded chromatograms. (Figs. 1B and 2)

The absorption spectra directly recorded from chromatograms within the range from 200 to 400 nm for spot coming from decomposition product (R_f 0.29) had similar shape and their maximum was slightly shifted in a direction of shorter wavelengths (λ_{max} 288 nm) in relation to spectra of moxifloxacin (λ_{max} 295 nm) (Fig. 3).

Based on these observations, it may be assumed that the same decomposition product with structure similar to moxifloxacin is generated independently on the composition of study solutions.

Faster decomposition of moxifloxacin was demonstrated during studies performed at 110°C. This decomposition was 52.04% after 48 h and increased in the presence of metal ions: Cu(II) (79.35%), Fe(III) (63.97%), Al(III) (62.42%) and Zn(II) (58.62%). The observed changes of moxifloxacin in the presence and absence of metal ions are similar to changes at 90°C. The fastest decomposition was observed in the presence of Cu(II) ions, and slower in case of Fe(III) to Zn(II) ions.

Besides increased decomposition of moxifloxacin, the observed changes also concern the number of generated decomposition products in the study. Similarly to studies at 90°C, a (P-2) peak with R_f 0.29 value and two extra peaks (P-1) and (P-3) with R_f 0.15 and R_f 0.89 values, respectively, were observed next to the studied compound on densitograms independently of the composition of the solution (Fig. 4)

The maxima of absorption spectra for decomposition products differ between themselves, P-1 (λ_1 275), P-2 (λ_{max} 288 nm), P-3 (λ_{max1} 281), and are slightly shifted towards shorter wavelengths in comparison to parent compound (λ_{max} 295 nm). In case of P-3 product, a well developed maximum of absorption at λ 340 nm is observed. Therefore, the structure of quinolinic ring is probably maintained and observable differences are only related to recpective substituents.

Kinetic and thermodynamic evaluation

For kinetic evaluation, results of moxifloxacin decomposition at 90 and 110°C were considered. It was established that decomposition of moxifloxacin in solutions in the presence and absence of metal ions proceeds according to kinetics of the first-order reaction based on changes of ln c = f(t) (Figs. 5 and 6).

The constant rate (k) for study solutions containing metal ions decreases in the following order: Cu(II) > Fe(III) > Zn(II) > Al(III), reaching the lowest values for moxifloxacin solutions in the absence

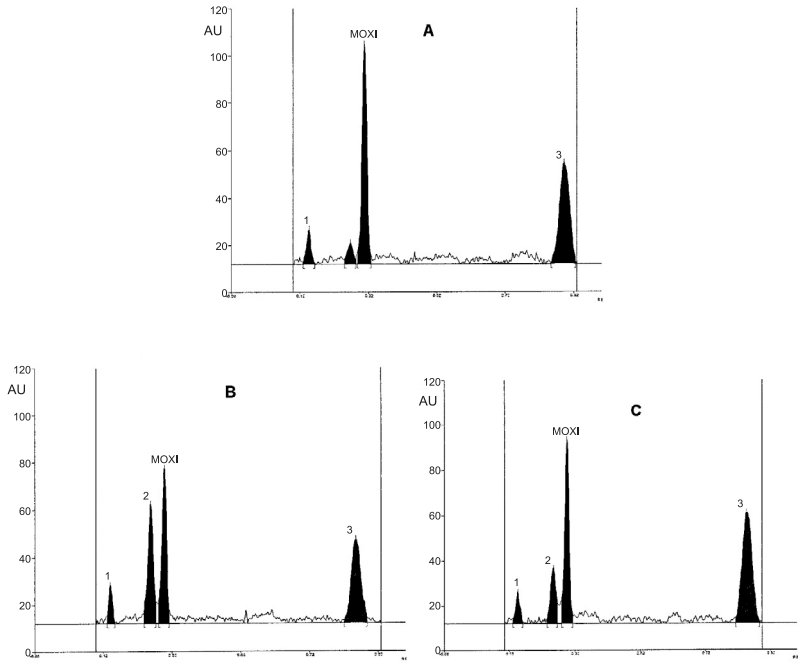

Figure 4. Densitograms of moxifloxacin hydrolysis in the absence (**A**) and in the presence of metal ions: Cu(II) (**B**) and Fe(III) (**C**) after 24 h heating at 110°C. Peaks: 1, 2, 3 the degradation products: P-1, P-2, P-3, respectively

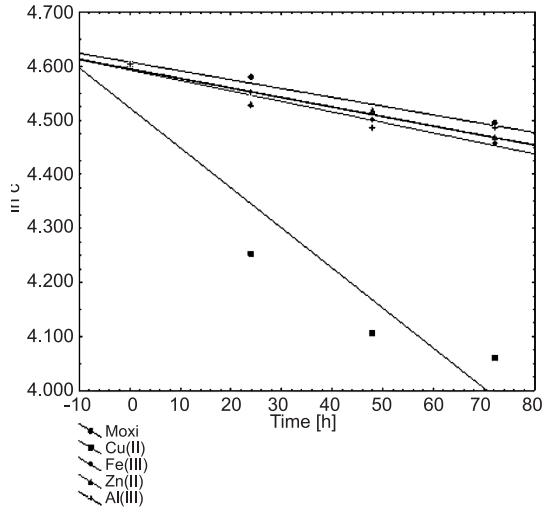

Figure 5. The ln c = f(t) graph of moxifloxacin in 3 mol/L hydrochloric acid at 90°C

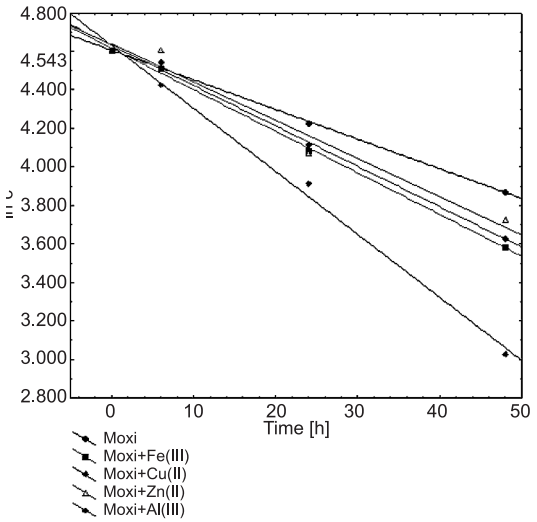

Figure 6. The ln c = f(t) graph of moxifloxacin in 3 mol/L hydrochloric acid at 110°C

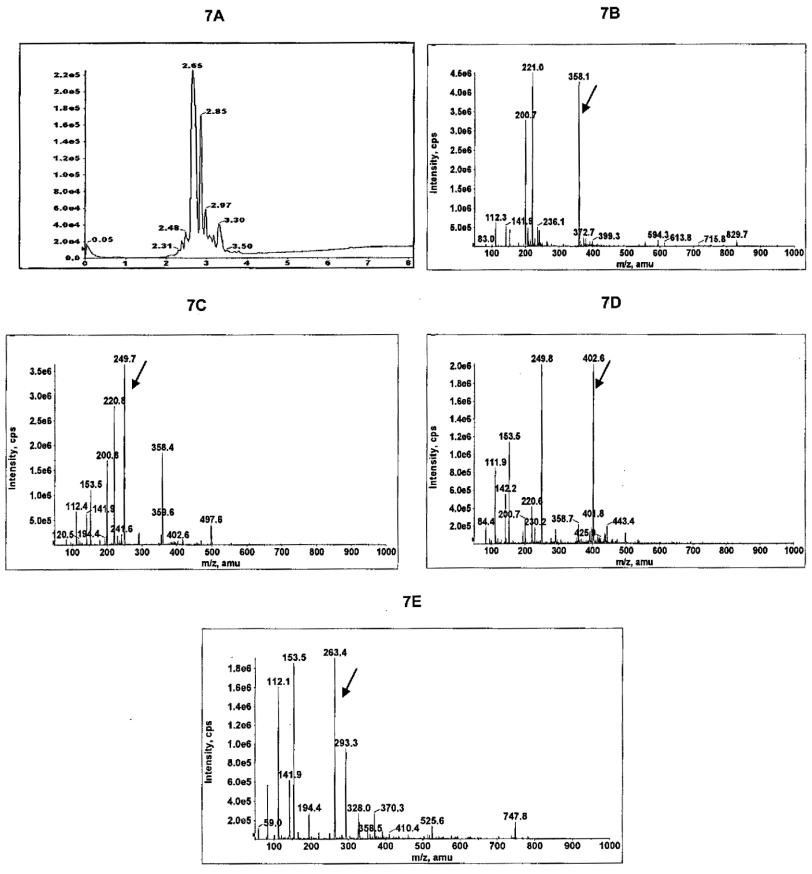

Figure 7. HPLC chromatogram with diode array detection of studied sample of moxifloxacin in acidic conditions with addition of Cu ions (**A**), mass spectrum of sample of studied drug at retention time of 2.65 min (**B**), 2.85 min (**C**), 2.97 min (**D**) and 3.4 min (**E**), respectively

of metal ions. The calculated values of $t_{0.1}$ and $t_{0.5}$ confirm better stability of moxifloxacin in solutions in the absence of metal ions, and the stability decreases in the presence of Zn(II), Al(III), Fe(III) ions and reaching the lowest durability in the presence of Cu(II) ions (Tab. 1).

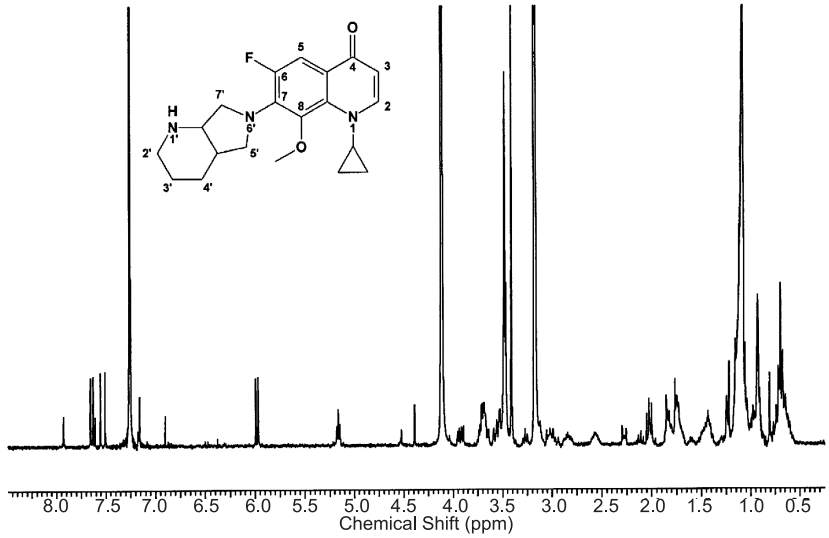

Figure 8. ^{1}H NMR spectrum of degradation product P-3

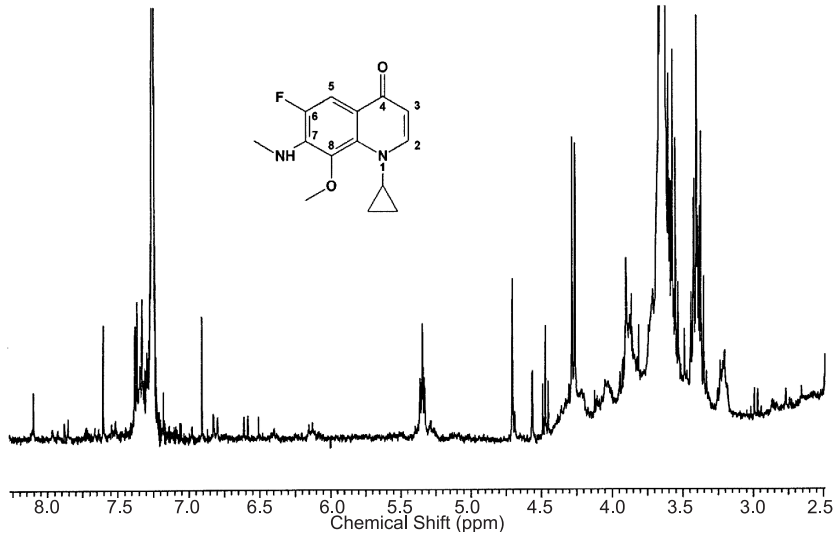

Figure 9. ^{1}H NMR spectrum of degradation product P-2

The calculated energy activation (E_a) value for hydrolysis process of mixofloxacin solutions in the presence of Cu(II) (E_a = 85.83 kJ/mol) is lower than values obtained in the presence of Fe(III) (E_a = 138.42 kJ/mol), Al(III) (E_a = 138.52 kJ/mol) and Zn(II) (E_a = 141.82 kJ/mol), and in the absence of metal ions ($E_{a\,Moxi}$ = 141.92 kJ/mol).

Based on the individual results of E_a and $t_{0.1}$ and $t_{0.5}$ parameters, some type of similarity may be observed, namely, both kinetic as well as thermodynamic parameters differ significantly in moxifloxacin solutions in the presence of Cu(II) ions, in which the fastest decomposition occurs, from values obtained for other solutions.

It seems that the effect of ions on decomposition of moxifloxacin and their varied contribution to the hydrolysis process, can be interpreted as a complex-generated activity of individual metal ions with quinolones, which results in formation of less or more solid bonds prone to decomposition.

Identification of hydrolysis products

LC-MS

The identification of degradation products of moxifloxacin and their structural analysis was done by LC-ESI/MS method. Three potential degradants were identified using the above mentioned procedure.

Figure 7A illustrates the HPLC diode array chromatogram of a sample of moxifloxacin under its degradation process in acidic environment with addition of cuprum ions at 110°C. The parent compound of moxifloxacin (m/z 402.6 amu) was observed at retention time of 2.97 min (Figure 7D). In the full scan mass spectrum of studied sample of moxifloxacin under the degradative analysis we observed at a retention time of 2.65 min a possible degradation product with m/z 358.1 amu, due to the loss of the functional carboxyl group from studied drug (Fig. 7B).

The next process could involve decarboxylation and hydrolysis of 2,8-diazabicyclo[4.3.0]non-8-yl group reaction of moxifloxacin, which gives the final product with m/z 263.4 amu observed at retention time 3.4 min (Fig. 7E). The similar reaction could be observed for degradation product at retention time of 2.85 min having m/z 249.7 amu (Fig. 7C).

^{1}H NMR

^{1}H NMR spectrum of sample P-3 shows characteristic signals corresponding to chemical structure of degradation product (Fig. 8).

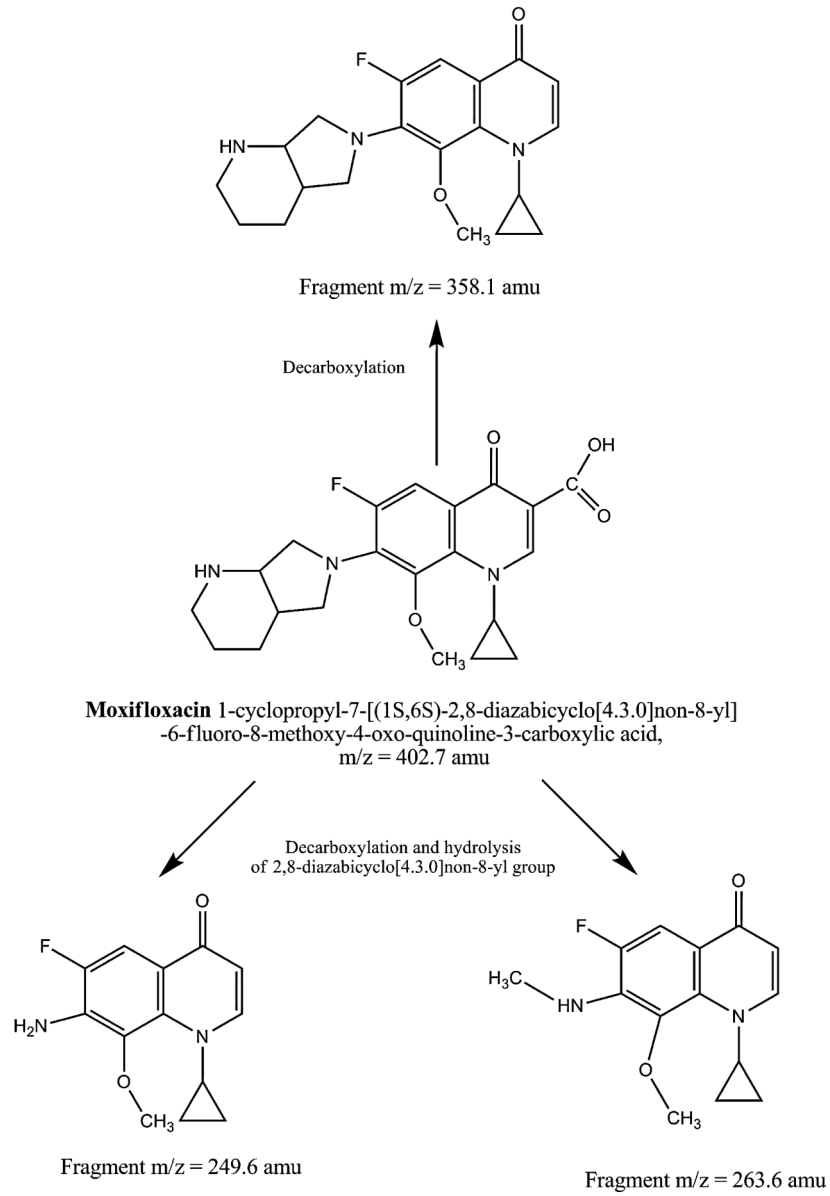

Figure 10. The plausible degradation patterns of moxifloxacin under studied conditions

The most important are signals of quinoline moiety protons, which indicates decarboxylation of starting compound. Two doublets δ 7.65 ppm (d, *J* = 7.7 Hz, H2) and δ 5.98 ppm (d, *J* = 7.7 Hz, H3) are clearly visible. The latter corresponds to proton in position of detached carboxylic group. In the aromatic fragment of spectrum there is one more doublet possessing large coupling constant: δ 7.53 ppm (d, *J* = 14.1 Hz, H5). This large value of coupling constant corresponds to H-C-C-F system. Confirmation can be found in ^{19}F spectrum, which exhibits only one signal: δ –123.58 ppm (d, *J* = 14.7 Hz) indicating fluorine atom coupled with one proton. Despite of complicated pattern of signals observed in the aliphatic region of the spectrum it is possible to conclude that there is no other changes in chemical structure of analyzed compound. Signals in the range 4–1 ppm can be assigned to protons of 2,8-diazabicyclo[4.3.0]nonane moiety and signals between 1 and 0.5 ppm can be assigned to protons of cyclopropyl fragment. There is also visible strong singlet at δ 3.41 ppm corresponding to protons of methoxyl group. Analysis of P-3 spectrum clearly indicates that on this stage of degradation process only decarboxylation occurs while the rest of molecule remains unchanged.

Due to very low concentration of investigated compound, analysis of its spectrum is possible only through the comparison to spectrum of sample P-3. It is worth to note that in the aromatic range of spectrum similar pattern of signal is observed (Fig. 9).

Three doublets at δ 7.65, 7.53 and 6.14 ppm indicate the same manner of substitution of quinoline moiety, which confirms decarboxylation process. Quite different set of signals observed in the aliphatic region of spectrum shows that degradation of bicyclic fragment occurs. Singlet at δ 3.38 ppm can be assigned to protons of methyl group yielded from this process. Apart of these changes, it is possible to find singlet assigned to methoxyl group protons at δ 3.41 ppm, indicating that hydrolysis process in this fragment of the starting compound does not occur. On that basis, it can be assumed that sample P-2 contains the product of subsequent decarboxylation and degradation of diazabicyclic moiety, which can be treated as a next step of moxifloxacin degradation.

The structure for P-1 spot could not be fully confirmed by ^{1}H NMR technique because of the low amount of received product and strong interferential background coming from the gel. However, based on the LC-MS results it may be assumed that the compound with m/z = 249.6 amu (P-1 spot) has a structure presented in Figure 10.

CONCLUSION

In this paper, a new method for determination of moxifloxacin in the presence of its hydrolysis products was developed. This method allows evaluation of hydrolysis process in the acidic environment in the studied concentration ranges. It was proved that stability of moxifloxacin depends on temperature, heating time, and the presence of metal ions in solution.

The highest impact on decomposition of moxifloxacin have Cu(II) ions. This impact decreases then in the following order Fe(III) > Al(III) > Zn(II).

Decomposition of moxifloxacin in the presence of metal ions proceeds according to the kinetics of the first-order reaction. The presence of metal ions in moxifloxacin solutions increases the rate of the reaction (k) and decreases activation energy (E_a), which is well exemplified in the case of Cu(II) ions. In the remaining solutions, independent on their composition, changes in the above parameters are not significant.

Based on the calculated $t_{0.1}$ and $t_{0.5}$ values, it can be assumed that decomposition of moxifloxacin is faster in the presence of ions. Under the described conditions, decomposition process of moxifloxacin results in development of three degradation products, which structures were determined by LC-MS and ^{1}H NMR techniques (Fig. 10).

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