



Trade Science Inc.

ISSN : 0974-7419

Volume 12 Issue 12

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(12) 2013 [461-466]

A validated stability indicating RP-LC method for determination of moxifloxacin in bulk powder and in pharmaceutical formulations

Mamdouh R.Rezk^{1*}, Iman A.Abdel-Karim²

¹Analytical Chemistry Department, Faculty of Pharmacy-Cairo University, Kasr El-Aini Street, ET-11562 Cairo, (EGYPT)

²Advanced Research Center (ARC), Nasr City, Cairo, (EGYPT)

E-mail : mamdoughreda75@yahoo.com

ABSTRACT

A simple stability indicating HPLC method was developed and validated for determination of moxifloxacin hydrochloride in the presence of its induced degradation products. The drug was subjected to stress stability studies including acidic, alkaline and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. The developed method utilized Symmetry C₁₈ column (250 × 4.6 mm, 5 μm) in an isocratic separation mode. The mobile phase consisted of methanol: 0.2 % triethylamine (pH 2.5 with orthophosphoric acid), (35: 65, v/v) at a flow rate 1.5 mL/min with UV-detection at 290 nm. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. The method was applied for determination of moxifloxacin hydrochloride in pure powder and in its pharmaceutical formulations.

© 2013 Trade Science Inc. - INDIA

KEYWORDS

Assay;
Moxifloxacin;
RP-HPLC;
Stability;
Validation.

INTRODUCTION

Moxifloxacin hydrochloride (MOX), is (1-cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride), Figure 1. It is a fluoroquinolone antibacterial agent^[1].

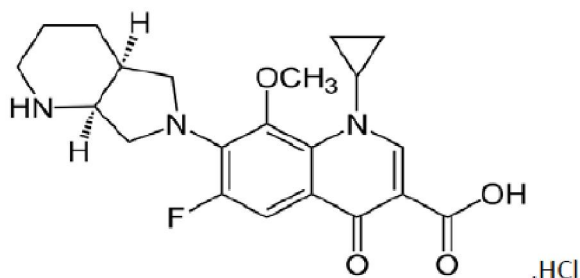


Figure 1 : Structural formula of moxifloxacin HCl.

In literature survey, some analytical methods including RP-HPLC^[2-4] and UV-spectroscopic^[5-7] and HPTLC^[8] methods have been reported for the estimation of MOX in bulk, pharmaceutical formulation and in biological samples.

In modern analytical laboratory there is always a need for simple and rapid method of analysis. The present work aimed to develop a fast, sensitive, selective and stability-indicating method for determination of MOX. The proposed method is able to selectively, determine MOX from its impurities, degradation products and placebo components. The developed method was validated with respect to specificity, linearity, limit of detection and quantification, precision, accuracy and robustness. Force degradation studies were performed on the placebo and drug product.

Full Paper

EXPERIMENTAL

Instruments

Agilent LC system (Böblingen, Germany) consisted of a quaternary pumping system (Agilent model G1311A), a UV variable wavelength detector (model G1315D, Agilent), and an auto sampler (model G13329A) equipped with a degasser (G1322A, Agilent).

Stationary phase consisted of a Symmetry C₁₈ analytical column (250 × 4.6 mm, 5 μm) (Waters, USA).

pH-meter (Mettler Toledo, Switzerland).

Materials and reagents

(a) Materials

- Moxifloxacin hydrochloride (MOX) pure powder was kindly supplied by Bayer Schering Pharma AG, (Leverkusen, Germany). Its purity was found to be 100.45±0.840 (n=6) according to the BP HPLC method^[1].

(b) Reagents

- Orthophosphoric acid: Adwic, El-Nasr Pharm. Co. (Cairo, Egypt). Methanol: HiPerSolv.[®], HPLC-grade, E. Merck (Darmstadt, Germany). Triethyl amine L R: Laboratory Rasayan s.d. Fine-Chem Ltd. De-ionized water: Bidistilled from Aquatron Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire, UK).

(c) Pharmaceutical formulations

- Avalox[®] film coated tablets, BN: BXG0DH1 (Bayer Schering Pharma AG, Leverkusen, Germany). Each film coated tablet claimed to contain 436.8 mg of moxifloxacin hydrochloride equivalent to 400 mg of free moxifloxacin.
- Vigamox[®] ophthalmic solution 0.5% as base, Lot.N.:203945F (Alcon, Laboratories, Inc., USA). Each mL claimed to contain 5.45 mg of moxifloxacin hydrochloride equivalent to 5 mg of free moxifloxacin.

(d) Standard solutions

All calculations were done regarding moxifloxacin free base.

(A) Stock standard solution of MOX

Stock standard solution of MOX (1mg/mL) was

prepared by transferring 109.1 mg of moxifloxacin hydrochloride powder (equivalent to 100 mg of MOX free base) into a 100-mL volumetric flask containing 50 mL distilled water. Shaking was done till complete dissolution then the volume was completed to the mark with water. Working solutions were prepared by serial dilutions.

Procedure

(a) Degradation of MOX

(A) Preparation of MOX alkaline degradates

A degraded sample of MOX was prepared by adding 25 mL of 2 molar NaOH to 54.7 mg of MOX hydrochloride (equivalent to 50 mg free MOX) and refluxing for 1 hr. The resulting solution was tested for complete degradation by thin layer chromatography using butanol: methanol: NH₃ (6:1:2, by volume) as a mobile phase and detecting the spots at 254 nm. The degraded solution was neutralized, transferred quantitatively into a 50-mL volumetric flask and brought to volume with water to prepare a stock solution of MOX alkaline degradates of 1 mg/mL.

(B) Preparation of MOX acidic degradates

The same procedure used for preparation of alkaline degradates was applied but a volume of 25 mL of 2 molar HCl was used instead of alkali. Reflux was done for 3 hours to achieve complete degradation of MOX as tested by the previously described thin layer chromatographic method.

(C) Preparation of MOX oxidative degradates

A degraded sample of MOX was prepared by adding 5 mL of 30% H₂O₂ to a 50 mL aqueous solution of MOX (1mg/mL) and then it was refluxed for 3 hours to achieve complete degradation. The resulting solution was tested for complete degradation by the thin layer chromatographic method described before.

(b) HPLC method

(A) Linearity

Aliquots (0.1-10mL) from MOX standard solution (1 mg/mL) were accurately transferred into a series of 100-mL volumetric flasks, the volume was then completed with the mobile phase to obtain a concentration range of 1-100 μg/mL.

Samples were then chromatographed using Sym-

metry C_{18} column (250 x 4.6 mm, 5 μ m) as a stationary phase. The mobile phase was formed of methanol: 0.2% triethylamine (pH 2.5 with *O*-phosphoric acid) (35:65, v/v). The flow rate was 1.5 mL/min, isocratically with UV detection at 290 nm. The injection volume was 10 μ L and column temperature was 35°C. The samples were filtered through a 0.45- μ m membrane filter. Analysis was usually performed after passing ~ 50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. Peak area ratio was plotted against concentration (using the area of 40 μ g/mL MOX solution as a divisor) to obtain calibration graph then the regression equation was computed.

The regression equation was used for estimating the concentration of MOX in pure samples, laboratory prepared mixtures with different degradates, unknown samples and in its pharmaceutical formulations.

Analysis of laboratory-prepared mixtures

Portions of MOX stock solution (1mg/mL) were transferred accurately into a series of 50 mL volumetric flasks. Aliquots from its corresponding acidic, alkaline or oxidative degradates solutions (each 1mg/mL) were added, separately, to prepare mixtures containing 10-90% of each degradation products. The prepared solutions were chromatographed by the described HPLC method and the concentration of MOX was computed from the regression equation.

Determination of MOX in its pharmaceutical formulations

(a) Avalox[®] tablets

At least 10 tablets were weight to determine the average weight of one tablet. A weight equivalent to one tablet was transferred into a 100- mL volumetric flask containing 50 mL methanol, sonicated for 15 minutes then diluted to volume with methanol. A volume of 1 mL of this solution was transferred into another 100-mL volumetric flask, then it was diluted with mobile phase to volume to obtain MOX solution having a concentration of 40 μ g/mL, and it was passed through a membrane filter with a 0.45 μ m porosity. The solution was injected to the LC system and the assay of MOX was done using the regression equation.

(b) Vigamox[®] ophthalmic solution

No sample treatment was done other than taking a

volume of 2 mL (equivalent to 10 mg MOX) into 10-mL volumetric flask containing 5 mL methanol, sonicated for 2 minutes then diluted to volume with methanol. A volume of 4 mL of this solution was transferred into another 100-mL volumetric flask, then it was diluted with mobile phase to volume to obtain MOX solution having a concentration of 40 μ g/mL, and it was passed through a membrane filter with a 0.45 μ m porosity. The solution was injected to the LC system and the assay of MOX was done using the regression equation.

Method validation

The developed method was fully validated according to ICH guidelines. Comparison of the results obtained by the proposed method and the official BP one was done in addition to statistical analysis of data.

RESULTS AND DISCUSSION

In this work, MOX degradation was performed using different stress conditions, namely; alkaline, acidic and oxidative ones. It was found that refluxing with 2M NaOH for one hour was sufficient for complete degradation of MOX as indicated by a thin layer chromatographic method using butanol: methanol: NH_3 (6:1:2, by volume) as a mobile phase and detecting the spots at 254 nm. The degraded solution was then neutralized, transferred quantitatively into a 50-mL volumetric flask and brought to volume with water to prepare a stock solution of MOX alkaline degradates of 1 mg/mL. Complete acidic degradation for MOX was achieved by refluxing with 25 mL of 2M HCl for 3 hours then neutralization was done by NaOH, while complete oxidation for MOX was achieved by refluxing with 5 mL of 30% H_2O_2 for 3 hours.

The International Conference on Harmonization (ICH) guidelines require the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance^[9]. An ideal stability indicating method is the one that quantifies the standard drug alone and also resolves its degradation products.

Method development

A simple isocratic high-performance liquid chromatographic method was developed for the determination of MOX in its pure form, in the presence of its

Full Paper

different degradates and in its pharmaceutical formulations. Different types of stationary phase C_8 and C_{18} columns with different dimensions and particle sizes were tested to find the best stationary-mobile phase match. It was found that Symmetry C_{18} (250 x 4.6 mm, 5 μ m) column gave the most suitable resolution for the separation of the drug from its degradation products. The mobile phase was chosen after several trials. The most suitable one was consisted of methanol: 0.2% triethylamine (pH 2.5 with *O*-phosphoric acid) (35:65, v/v). The flow rate was 1.5 mL/min and UV detection at 290 nm. The injection volume was 10 μ L and column temperature was 35°C. The samples were filtered also through a 0.45- μ m membrane filter. Analysis was performed after passing ~ 50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. Peak area ratio was plotted against concentration (using the area of 40 μ g/mL MOX solution as a divisor) to obtain calibration graph then the regression equation was computed. System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor, and resolution.

By using the described chromatographic conditions, MOX was well separated from its degradants with average retention time of 6.4 min., Figures 2 (a, b, c & d).

Peak purity was confirmed for the intact MOX peak using the photodiode array detector.

Method validation

Under the specified optimum chromatographic conditions, a calibration graph was constructed by plotting the relative peak area versus the corresponding concentration of MOX in μ g/mL. Linearity range was found to be 1–100 μ g/mL for MOX using the following regression equation:

$$A = 0.0248 C + 0.0056, r = 0.9999.$$

Where, A = the relative peak-area ratio (using the 40 μ g/mL of MOX as a divisor),

C = the concentration of MOX (μ g/mL)

and r = the correlation coefficient.

The mean percentage recoveries of pure samples were found to be 100.22 ± 0.635 (n=6). Validation results are represented in TABLE 1. Statistical comparison showed no significant difference between the developed method and the official one^[1] as shown in TABLE 2 which revealed that the calculated t-test and

F-value are less than the tabulated ones.

TABLE 1 : Assay parameters and validation sheet for the developed HPLC method.

Parameters	The developed HPLC method
Range (μ g/mL)	1-100
Regression equation	
Slope	0.0248
Intercept	+ 0.0056
Correlation coefficient	0.9998
Accuracy	
Mean \pm SD	100.22 ± 0.635
Precision	
Intra-day (Mean \pm SD)	100.05 ± 0.431
Inter-day (Mean \pm SD)	99.97 ± 0.720

TABLE 2 : Statistical comparison for the results obtained for MOX by the proposed method with the HPLC official one^[1].

Parameter	The proposed HPLC method	Official method ^[1]
Mean	100.22	100.45
SD	0.635	0.840
Variance	0.403	0.706
n	6	6
F-test	1.75 (5.05)*	
Student's t- test	0.535 (2.228)*	

* The values in the parentheses are the corresponding theoretical t- and F-values at $P = 0.05$.

The robustness of the HPLC method was examined by the analysis of samples under a variety of experimental conditions such as small changes in the pH (± 0.2 units), small changes in proportions of mobile phase, by up to $\pm 2\%$. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.

Stability indication

To assess the stability-indicating efficiency of the proposed method, MOX was mixed with its alkaline, acidic or oxidative degradants, separately, in different ratios and analyzed by the proposed method. TABLE 3 illustrates good selectivity in the determination of MOX in the presence of up to 90% (w/w) of its corresponding degradants. The suggested method was successfully applied for the determination of MOX in its phar-

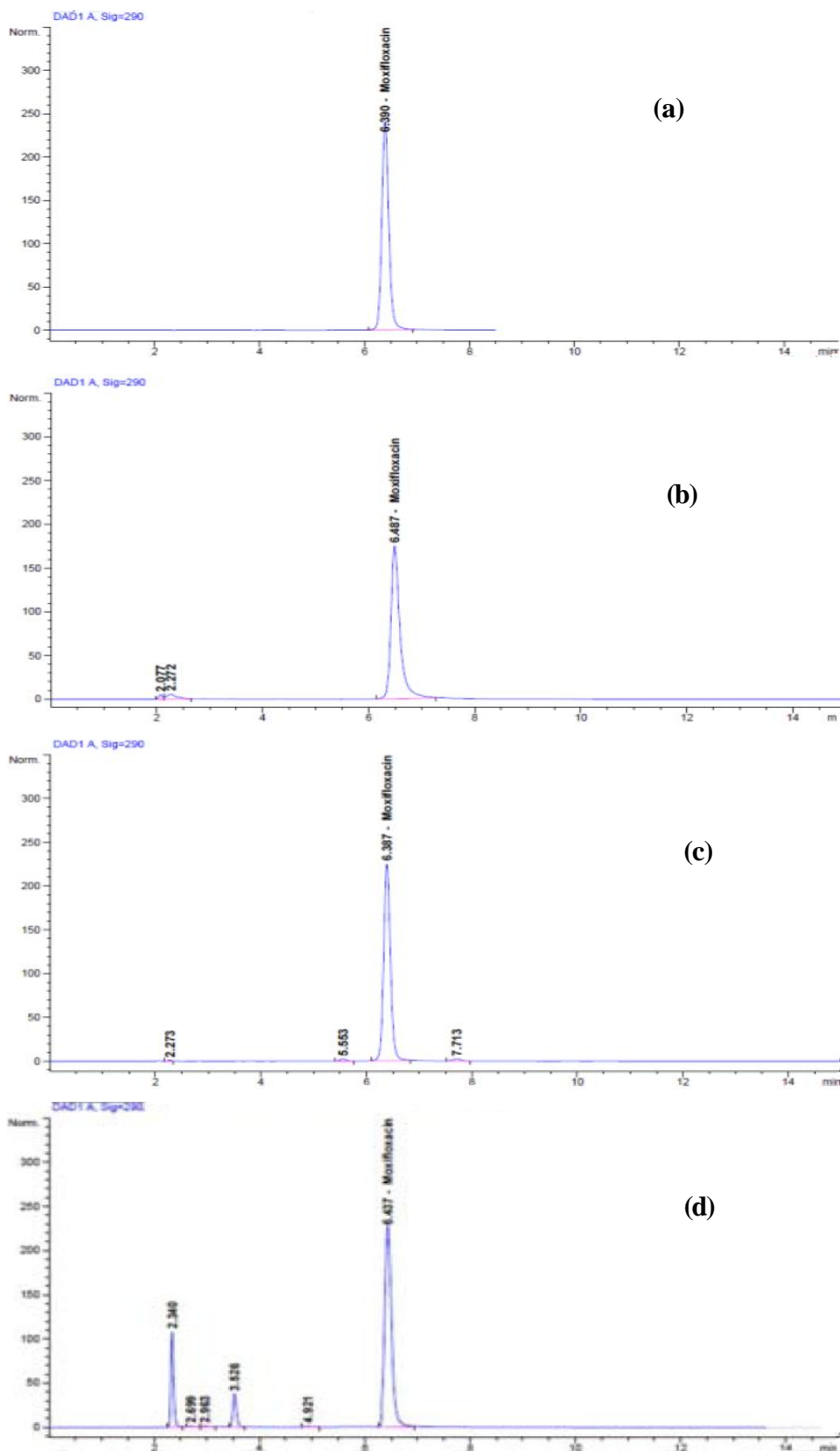


Figure 2 : HPLC chromatogram for the developed method under the specified conditions showing. (a) MOX (40 $\mu\text{g/mL}$) (R_t : 6.39 min.); (b) Laboratory prepared mixture of MOX and its alkaline degradates; (c) Laboratory prepared mixture of MOX and its acidic degradates; (d) Laboratory prepared mixture of MOX and its oxidative degradates.

Full Paper

maceutical formulations, with good percentage recoveries, TABLE 4.

TABLE 3 : Determination of MOX in laboratory prepared mixtures with its degradants by the proposed method.

Laboratory prepared mixture*	The proposed HPLC method
MOX with its alkaline degradates (Mean \pm SD)	100.19 \pm 0.825
MOX with its acidic degradates (Mean \pm SD)	99.92 \pm 0.538
MOX with its oxidative degradates (Mean \pm SD)	100.58 \pm 0.744

* In the presence of up to 90 % (w/w) degradants content

TABLE 4 : Determination of MOX in its pharmaceutical formulations by the proposed method.

Preparation	The proposed HPLC method
Avalox[®] film coated tablets	
BN: BXG0DH1	
(Mean \pm SD)	101.09 \pm 0.915
Vigamox[®] ophthalmic solution 0.5% as base	
Lot.N.: 203945F	
(Mean \pm SD)	99.78 \pm 0.833

CONCLUSION

The suggested method is found to be simple, accurate, selective, and equally sensitive with no significant difference of the precision compared with the official HPLC method of analysis^[1]. Application of the proposed method to the analysis of MOX in its pharmaceutical formulations shows that neither the excipient nor the degradation product interferes with the determination. The run time is relatively short, which enable rapid determination of many samples in routine and quality control analysis.

REFERENCES

- [1] British pharmacopoeia seventh ed., The stationary office medicinal and pharmaceutical substances, London, (2012).
- [2] A.L.Djurdjevic, M.J.Stankov, P.Djurdjevic; Journal of Chromatography B, **844**, 104-111 (2006).
- [3] P.Djurdjevic, A.Ciric, M.Stankov, A.Djurdjevic, M.J.Stankov; J.Pharm.and Biomed.Anal., **50**, 117-126 (2009).
- [4] S.T.Ulu; J.Pharm.and Biomed.Anal., **43**, 320-324 (2007).
- [5] M.A.Sultan; Arabian Journal of Chemistry, **2**, 79-85 (2009).
- [6] S.K.Motwani, S.Chopra, F.J.Ahmad, R.K.Khar; Spectrochimica Acta Part A, **68**, 250-256 (2007).
- [7] M.Misra, A.K.Misra, G.M.P.Zope, A.K.Dorle; Journal of Global Pharma.Technology, **2**, 21-27 (2010).
- [8] S.K.Motwani, R.K.Khar, F.J.Ahmad, S.Chopra, K.Kohli, S.Talegaonkar; Analytica Chimica Acta, **582**, 75-82 (2007).
- [9] ICH, Stability testing of new drug substances and products international conference on harmonization, Geneva, (1993).