MUTUAL SPECTROPHOTOMETRIC DETERMINATION OF MOXIFLOXACIN DRUG AND IRON(III) IONS BY FORMATION OF A COMPLEX COMPOUND

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A simple, new and sensitive spectrophotometric method has been developed for the quantitative determination of the drug Moxifloxacin HCl (MOX), in pure form and in pharmaceutical formulation; and Fe(III) ions. The method was based on the formation of a colored complex between the drug and Fe(III) ions. The maximum absorption wavelength was 450 nm for determination of both MOX and Fe(III) ions. Beer's law was obeyed in the concentration range of 14.45 – 394.2 μg mL^{-1} (r^2 = 0.998) for determination of MOX and 2.8 – 22.4 μg mL^{-1} (r^2 = 0.97) for determination of Fe(III) ions. The conditions for complex formation were studied and optimized to obtain the highest absorbance available. The method was successfully applied for the analysis of commercial tablets (Maxim), and the recovery study reveals that there is no interference from the common excipients that are present in tablets. The results obtained by the proposed method were compared with that obtained by a standard reference one. Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-test at 95% confidence level. The results proved that there no any significant difference, regarding accuracy and precision, between the two compared methods.

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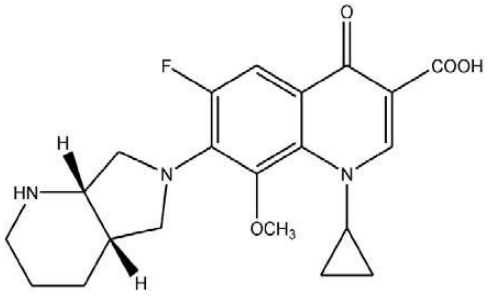
Introduction

Moxifloxacin (MOX) (Scheme 1) is a new fourth generation 8-methoxy fluoroquinolone. Its chemical name is [1-cyclopropyl-7-(S,S)-2,8-diazabicyclo(4.3.0)-non-8-yl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid hydrochloride]. The drug was developed primarily for the treatment of community acquired pneumonia and upper respiratory tract infections. It is active against Gram negative pathogens, Gram positive cocci, aerobic intracellular bacteria, atypical organisms and anaerobic bacteria[1]. It has an empirical formula $C_{21}H_{24}FN_3O_4$. HCl with molecular weight 437.89. The drug is an odorless yellow crystalline powder with a melting point in the range 324 – 325 °C. The pH of its 0.1% aq. solution is 4.0 - 5.0. The drug base is sparingly soluble in water, slightly soluble in ethanol (96%) and practically insoluble in acetone; but the hydrochloride form of the drug is fairly soluble in water[2].

Only a little is available in the literature about the analytical determination of MOX, this is may be attributed to the relatively recent abundance of the drug. However, several methods are known for quantitative determination of MOX, including spectral[1,3-12], chromatographic[13-25] and electroanalytical techniques.[9,22,26-29]

The chromatographic method for the determination of MOX requires an automated system, which is not available in many research laboratories. Therefore, it was considered worthwhile to develop rapid and sensitive procedures suitable for the routine quality control analysis of the investigated drug. Spectrophotometric methods still belongs to the most frequently used analytical techniques in pharmaceutical analysis, which gives practical and significant economic advantages over other methods. So, the present study is an investigation and developing a new spectrophotometric method for the determination of MOX in pure form and in its pharmaceutical formulation; and Fe(III) ions, based on the formation of a colored complex compound.



Scheme 1. Chemical structure of moxifloxacin (neutral form).

Experimental

Materials and reagents

All chemicals used in the present investigation were of the highest purity grade chemicals. Ferric oxide was obtained from Sigma-Aldrich products and ferric chloride was obtained from Fluka. Pure MOX was provided by Jamjoom Pharma, Jeddah, KSA. MOX tablets (Maxim) was produced

by Jamjoom Pharma and purchased from local market. The water used for all preparations and washing was freshly prepared deionized water.

Instrumentation

Perkin-Elmer precisely model Lambda 25 – USA, UV-VIS spectro-photometer was used for all spectrophotometric measurements by using quartz cells of 1-cm optical path length. pH - Bench meter model Martini instruments - Romania was used for pH measurements. Millipore (Elix 10) deionizer – USA was used for obtaining deionized water.

Preparation of standard and sample solutions

Preparation of stock standard solution

Stock solution of moxifloxacin.HCl (0.01 M) was prepared by dissolving the accurately weighed amount of the pure drug in the least amount of water, heating until the powder is completely dissolved, then completed to the volume with deionized water. Dilute solutions of the drug were prepared by accurate dilution of the stock 0.01 M solution with deionized water to obtain working concentrations in the range of 14.45 – 394.2 μg mL^{-1}.

Preparation of dosage forms sample solution

Five tablets of MOX drug (Maxim) were ground and mixed well to a fine homogenous powder. An accurately calculated amount of the powder to prepare stock (0.01 M) solution was weighed and dissolved in deionized water with vigorous shaking until complete dissolving of the drug. The solution was centrifuged, filtered, transferred quantitatively to a measuring flask, and completed to the volume with deionized water and shaked well. The solution was standardized spectrophotometrically[4]. Dilute solutions were prepared by accurate dilution whenever needed.

General procedures for determination of MOX

For the determination of MOX using Fe(III) ions, a large excess of Fe(III) ions was added to the drug solution. The absorbance of the formed complex solution was measured at 450 nm against a blank prepared from the same amount of the drug. A calibration curve is constructed between the concentration of the drug and absorbance. All measurements were carried out at room temperature.

General procedures for determination of Fe(III) ions

For the determination of Fe(III) ions using MOX, a large excess of MOX was added to the Fe(III) ions solution. The absorbance of the formed complex solution was measured at 450 nm against a blank prepared from the same amount of the drug. A calibration curve is constructed between the concentration of Fe(III) ions and absorbance. All measurements were carried out at room temperature.

Stoichiometry

The stoichiometric ratios of the formed complexes were determined either by continuous variation or molar ratio methods. In continuous variation method[30], a series of solutions was prepared by mixing equimolecular amounts ($1x10^{-3}$ M) of the Fe(III) ions and drug in varying proportions, while keeping the total molar concentration constant. A plot of the absorbance at the recommended wavelength versus the mole fraction of the drug gave a maximum at the molar ratio of the formed complex. In molar ratio method[31], a series of solutions was prepared by mixing a constant concentration of the drug (3×10^{-4} M and 4×10^{-4} M) with varying concentrations of the Fe(III) ions, then completed with deionized water up to 10 mL. The absorbances of these solution were measured, then plotted versus the ratio between concentration of the iron and total concentration. The plots are straight lines intersect at the most probable molar ratio of the formed complex.

Determination of formation constant for the complex

The formation constant (K_n) of the complex was determined by substituting the data of continuous variation in following equation[32]:

$$K_n = \frac{\frac{A}{A_m}}{\left[\frac{1-A}{A_m}\right]^{n+1} C^n n^n} \qquad (1)$$

where A is the absorbance of the complex at concentration C of the drug, A_m is the maximum absorbance of the complex at full color development which can be obtained from Job's continuous variation curve, and n is the stoichiometric ratio between drug and Fe(III) ions.

Results and discussion

Investigations were carried out to establish the optimal conditions leading to a maximum color development for the quantitative determination of MOX and Fe(III) ions.

Selection of the maximum wavelength

When the spectra of the complex were scanned against deionized water or Fe(III) ions as a blank, it was observed that there is a great absorption in the range 420 – 460 nm; this new absorption may be attributed to the formation of a complex between the drug and Fe(III) ions. The new band of the complex may be obscured by the highly sensitive shoulder of the drug at 360 nm. So, the spectra of the complex were recorded against the pure drug itself as a blank. The results are shown in Fig. 1. It can be shown that a new band at 450 nm appears clearly in this case without any interference from other bands of the drug itself. This new band may be attributed to the formation of a complex between MOX and Fe(III) ions. So, we decided that all of the subsequent measurements will be performed by using the same amount of the drug as a blank.

Effect of solvent

The spectra of the complex were scanned in three solvents; water, acetone and methanol, respectively. The results showed that the absorbance increases slightly in case

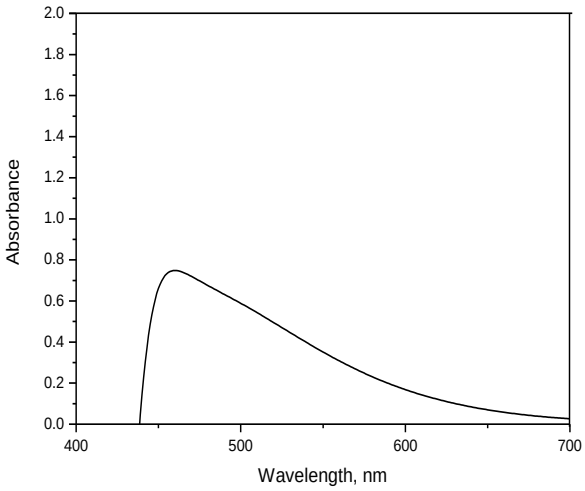


Figure 1. Absorption spectra of the complex in aqueous media against MOX solution as a blank

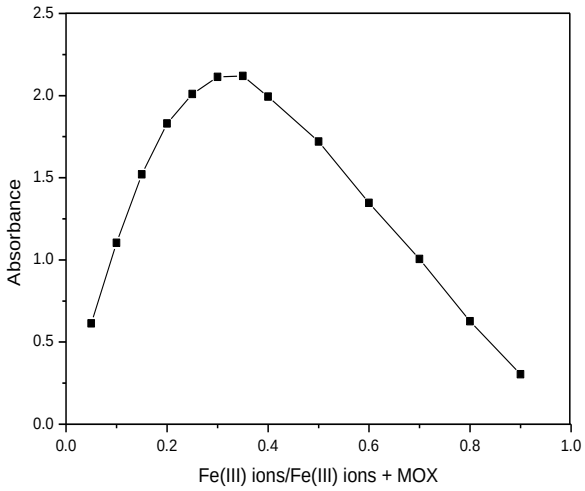


Figure 2. Job's continuous variation method of MOX – Fe(III) ions

of acetone or methanol. But the differences between them and water are not so large. So, all subsequent measurements are performed in aqueous solutions, as water is a widely popular solvent.

Effect of time and temperature

The effect of time on the formation of the complex at room temperature was investigated by allowing the reaction to proceed at different time intervals. The results revealed that the reaction went to completion instantaneously and longer reaction time did not affect the absorbance values. In addition the stability of the complex was studied by following its absorption intensity at different time intervals. The results showed that the absorbance remains stable for more than 48 hours.

The effect of temperature on the reaction and stability of the complex was studied by carrying out the reaction in aqueous medium at different temperatures (25 - 70 ºC). The results revealed that there is no effect of temperature on the reaction of the complex formation.

Effect of pH

The influence of pH on the absorbance of the studied complex was investigated by adding varying amounts of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide to the prepared complex solution. The results revealed that the absorbance in the acidic solutions (pH 2.0 – 3.0), prepared by addition of HCl or without any additions, are very close to each other. Addition of sodium hydroxide with small concentrations (up to pH 3.5), also produces the same absorbance. So, the subsequent measurements are carried out at pH 2 – 3.5 (in presence of the complex only, without addition of neither HCl nor sodium hydroxide).

Stoichiometry of the complex

Under the optimum conditions, the stoichiometry of the reaction between moxifloxacin HCl and Fe(III) ions was investigated by Job's method[30] and molar ratio method[31]. The bell shape of Job's plot (Fig. 2) indicated that the MOX : Fe(III) ions ratio was 2:1.

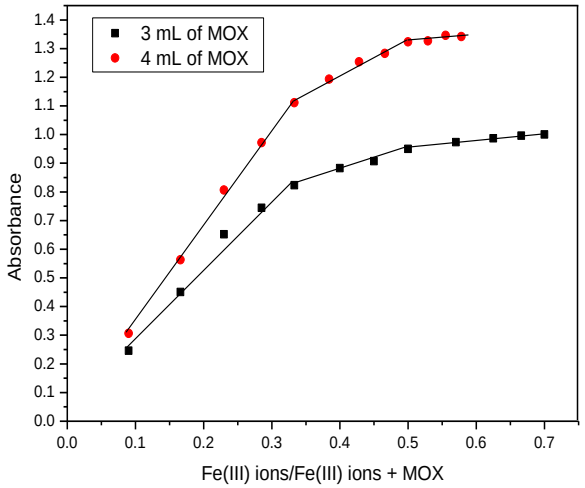


Figure 3. Molar ratio method of MOX – Fe(III) ions complex in aqueous media

The molar ratio method plot (Fig. 3) indicated that the MOX : Fe(III) ions ratio was 2:1 and 1:1.

The Formation Constant of the complex and Free Energy of complexation

The formation constant (K_n) of MOX – Fe(III) ions complex was calculated from the continuous variation data using Eq. (1). The value of K has been determined at several concentration of the drug and the mean value is calculated to be (5.5 ± 0.33) * 10^6 (L $mole^{-1})^2$ (K_{mean} ± SD). It is shown that the value of K is highly enough for complete formation of the complex and to be used in quantitative analysis.

The standard free energy of complexation ΔG^o is related to the formation constant by the following equation:

$$\Delta G^o = -2.303\ RT \log K \qquad (2)$$

where ΔG^o is the free energy change of the complex; R is the general gas constant (1.987 cal mol^{-1} $degree^{-1}$); *T* is the temperature in Kelvin; and K is the formation constant of MOX – Fe(III) ions complex (L mol^{-1}).

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Table 1. Analytical parameters for determination of both MOX using Fe(III) ions, and Fe(III) ions using MOX

Parameter	Determination of MOX	Determination of Fe(III)
λ_{max} (nm)	450	450
Beer's law, µg mL^{-1}	14.45 – 394.2	2.8 – 22.4
Molar absorptivity, L mol^{-1}cm^{-1}	5.74 x 10^{3}	5.46 x 10^{3}
Specific absorptivity, L g^{-1} cm^{-1}	6.12	5.86
Sandell sensitivity, µg cm^{-2}	1.63 x 10^{-4}	1.7 x 10^{-4}
Detection limit (DL), µg mL^{-1}	4.34	1.68
Quantification limit (QL), µg mL^{-1}	14.45	2.8
Ringbom range, µg mL^{-1}	21.9 – 175.2	2.8 – 11.22
Slope of the regression line	6.55 x 10^{-3}	9.77 x 10^{-2}
Intercept of the regression line	-5.5 x 10^{-2}	0.100
Correlation coefficient (r^2)	0.998	0.97
SD of the regression line	0.048	0.227

The value of ΔG^o is equal to (-9.19 ± 0.003) kcal mole^{-1} (ΔG^o ± SD). The negative value of ΔG^o indicates that, the reaction between the drug and Fe(III) ions is spontaneous.

Calibration curve and sensitivity

Under optimum conditions, standard calibration curves for determination of MOX using Fe(III) ions, and for determination of Fe(III) ions using MOX were constructed. The molar absorptivity, specific absoptivity, Sandell's sensitivity and correlation coefficient were determined. Detection limit (DL) and quantification limit (QL)[33] were determined for validation of the analytical method. The regression equation for the proposed procedures are derived using the least squares method and the correlation coefficient was 0.998 for determination of MOX and 0.97 for determination of Fe(III) ions. Table 1 shows the different analytical parameters obtained for determination of MOX and Fe(III) ions.

Reproducibility

The reproducibility of the proposed method was evaluated by performing four replicate analyses of varying concentration covering Beer's law range. The standard deviation and coefficient of variation were determined. The results are show in Table 2 for determination of MOX and Table 3 for determination of Fe(III) ions.

Table 2. Evaluation of precision of the proposed method for determination of MOX using Fe(III) ions

Concentrations, µg mL^{-1}	SD[a]	C.V.[b]
21.9	1.609	8.07
43.8	0.623	1.76
87.6	2.53	3.37
131.4	0.666	0.58
175.2	0.75	0.47
219	3.68	1.82
262.8	0.841	0.34
306.6	0.53	0.18
350.4	2.00	0.593
394.2	1.24	0.327

a: Standard deviation for four replicate measurements;
b: Coefficient of variation for four replicate measurements.

Table 3. Evaluation of precision of the proposed method for determination of Fe(III) ions using MOX

Concentrations, µg mL^{-1}	SD[a]	C.V.[b]
2.8	0.075	2.66
5.6	0.174	2.86
11.2	0.035	0.27
16.8	0.104	0.52
22.4	0.293	1.12

a: Standard deviation for four replicate measurements.
b: Coefficient of variation for four replicate measurements.

Application to Pharmaceutical Preparations

It is evident from the above mentioned observations that the proposed method gave satisfactory results with MOX in pure solution. Thus, its pharmaceutical dosage (Maxim tablets) was subjected to the analysis of its MOX contents by the proposed and a previously reported method[4], which is based on direct measurement of the absorbance of the formed metal complex.

The recovery percentages, using the proposed method, are within the range 100.07 ± 0.52 to 101.99 ± 0.24 (Table 4). These results were compared with that obtained from the previously reported method for pharmaceutical formulation (Table 4). The statistical comparison has been performed with respect to the accuracy (*t*-test) and precision (*F*-test)[34] of the two methods. It was found that there is no significant difference between the calculated and theoretical values of (*t*) and (*F*) at 95% confidence level. This indicated similar precision and accuracy in the analysis of MOX by both methods.

Moreover, to check the validity of the proposed method, the standard addition method was applied by adding small increments of standard MOX to a previously analyzed Maxim solution. The recovery results calculated by comparing the concentrations obtained from the spiked mixtures with those obtained by the reference method[4] were depicted in Table 5. The table, also, includes the standard deviation of the regression lines. The results confirm that the proposed method is not liable to interference by tablet fillers as well as the possibility of determination of lower concentrations using standard addition method.

Table 4. Determination of MOX in pharmaceutical formulation (Maxim tablets) using calibration curve method.

Taken, µg	Found, µg	Recovery±RSD%	F-test[a]	SD	C.V.
14.25	14.45	101.4±4.6	9.54	0.66	4.6
57.8	58.7	101.55±2.74	1.95	1.6	2.74
116.9	117	100.08±0.87	5.13[b]	1.02	0.87
154.2	156.2	101.29±3.14	3.15	4.9	3.14
192.7	194.3	100.83±1.7	1.44	3.32	1.7
270	270.2	100.07±0.52	3.83	1.41	0.52
308.3	312	101.2±1.72	3.78	5.37	1.72
346.9	348.3	100.4±0.46	3.01	1.59	0.46
366.2	371.3	101.39±0.35	4.5	1.3	0.35
385.4	393.1	101.99±0.24	8.94	0.92	0.24

RSD%: relative standard deviation of three replicate analyses except for (b) relative standard deviation of seven replicate analyses; a: Theoretical value for *F* is 19 for two degrees of freedom and 95% confidence limit; b: Theoretical value for *F* is 5.14 for six degrees of freedom for the proposed method and two degrees of freedom for the reference method and 95% confidence limit.

Conclusion

The proposed method for determination of MOX using Fe(III) ions is beneficial over many of the reported methods due to its sensitivity, accuracy, high percentage of recovery, wide application range, low relative standard deviation, and also due to the fact that it doesn't need expensive sophisticated apparatus as the measurements are carried out in the visible region of the spectra. Furthermore, the Fe(III) ions are not expensive and are available in all analytical laboratories. Therefore, the method is practical and valuable and can be used for routine application in quality control laboratories for analysis of MOX. Also, the method can be applied for determination of Fe(III) ions accurately and precisely.

Table 5. Determination of MOX in pharmaceutical formulation (Maxim tablets) using standard addition method

Recovery±SD%[a]	Found, µg	Taken, µg
98.31±0.04	7.01	7.13
101.96±0.03	14.53	14.25
100.1±0.02	57.86	57.8
98.6±0.02	115.26	116.9
100.06±0.01	192.83	192.7

a: SD for regression line.

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