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ORIGINAL ARTICLE

New, simple and validated kinetics spectrophotometric method for determination of moxifloxacine in its pharmaceutical formulations

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Abstract The objective of this research was to develop a kinetic spectrophotometric method for determination of moxifloxacine (MOXF) in pure form and pharmaceutical formulations. The method was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of MOXF by its reaction with 2,3,5,6-tetrachloro-1,4-benzoquinone in presence of acetaldehyde.

The formation of the colored product was monitored spectrophotometrically by measuring the absorbance at 652 nm. Factors affecting the reaction were studied and optimized. The stoichiometry of the reaction was determined, and the reaction pathway was postulated. The activation energy of the reaction was calculated and found to be 6.65 kJ mol^{-1}. Under the optimized conditions, the initial rate and fixed time (at 5 min) methods were utilized for constructing the calibration graphs. The graphs were linear in concentration ranges 5–100 and 15–150 μg ml^{-1} with limit of detection of 2.0 and 5.0 μg ml^{-1} for the initial rate and fixed time methods, respectively. The analytical performance for both methods was fully validated, and the results were satisfactory. No interference was observed from the excipients that are commonly present in the pharmaceutical formulations. The proposed method was successfully applied to the determination of MOXF in its pharmaceutical formulations. The label claim percentages were 101.25 ± 0.73% and 100.92 ± 0.65% for the initial rate and fixed time method, respectively. Statistical comparison of the results with those obtained by a reference spectrophotometric method showed excellent agreement between the accuracy and precision of the two methods. The proposed method has great value in its application to the analysis of MOXF in quality control laboratories.

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1. Introduction

Moxifloxacin (MOXF) {1-cyclopropyl-7-[2,8-diazobicyclo (4.3.0) nonane]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinolone carboxylic acid} is a new 8-methoxyquinolone derivative of fluoroquinolones with enhanced activity in vitro against

Gram-positive bacteria and maintenance of activity against Gram-negative bacteria (Balfour and Lamb, 2000; Vishwanathan et al., 2002; Biedenbach et al., 1998). The drug is rapidly absorbed, reaching maximum plasma concentrations between 1 and 4 h after oral administration; its half life of 11–15 h allows a daily administration (Biedenbach et al., 1998).

Moxifloxacin (MOXF) is administered to patients in 400 mg daily doses, being that the final concentrations in serum and urine for the treated patients are of 2.00–5.00 and 30.00–60.00 μg ml^{-1}, respectively (Wise et al., 1999). MOXF is prescribed for the bacterial infections of the respiratory tract including sinusitis, community acquired pneumonia and acute exacerbation of chronic bronchitis (Muijsers and Jarvis, 2002). Due to clinical advantages of MOXF, there has been increase in number of MOXF formulations in market in recent past.

No official method for determination of MOXF in pharmaceutical products is described in the pharmacopoeia. A survey of literature revealed that MOXF has been determined in biological fluids or pharmaceutical products by: high-performance liquid chromatography (HPLC) (Vishwanathan et al., 2002; Chan et al., 2006; Uiu, 2007; Kumar and Ramachandran, in press), voltammetry (Trindade et al., 2005), capillary electrophoresis with laser-induced fluorescence (Faria et al., 2006), atomic absorption (Al-Ghannam, 2008), spectrofluorometry (Ocana et al., 2000) and spectrophotometry (Al-Ghannam, 2008; Motwani et al., 2007; Venugopal and Saha, 2005; Patel et al., 2005; Ilango et al., 2006; Jane et al., 2006).

The chromatographic, voltammetric, electrophoretic and atomic absorption methods utilized delicated and/or expensive instruments that are not available in most quality control laboratories. Spectrophotometric technique is the most widely used in pharmaceutical analysis (Darwish et al., 2008, 2009). The widespread of spectrophotometric methods is attributed to their inherent simplicity, economic advantages and wide availability in most quality control laboratories.

However, few spectrophotometric methods were reported for determination of MOXF in its pharmaceutical dosage forms (Al-Ghannam, 2008; Motwani et al., 2007; Venugopal and Saha, 2005; Patel et al., 2005; Ilango et al., 2006; Jane et al., 2006). These methods were associated with some major drawbacks such as decrease selectivity due to measurement in ultraviolet region (Venugopal and Saha, 2005) and/or decreased simplicity of the assay procedure (e.g. tedious precipitation (Al-Ghannam, 2008)).

For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of MOXF in its pharmaceutical dosage forms. Kinetic spectrophotometric methods are becoming of great interest in the pharmaceutical analysis (Chamjangali et al., 2006; Darwish, 2005; Rahman et al., 2006). The application of these methods offers some specific advantages such as improved selectivity due to the measurement of the evolution of the absorbance with the reaction time. As well, it proved the avoiding of the interference of the colored and/or turbidity background of the samples, and possibility the interference of the other active ingredients present in the combined pharmaceutical formulations.

No attempts have been reported for the kinetic spectrophotometric determination of MOXF. The present study describes, for the first time, the development and validation of a selective and simple kinetic spectrophotometric method for the determination of MOXF.

The proposed method was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of MOXF by its reaction with 2,3,5,6-tetrachloro-1,4-benzoquinone (TCBQ) in presence of acetaldehyde. The development of the color was monitored spectrophotometrically at its maximum absorption peak. The initial rate and fixed time methods, after their full optimization and validation, were adopted for the determination of MOXF in its pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

Double beam V-530 (JASCO Co. Ltd., Kyoto, Japan) ultraviolet–visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements.

2.2. Chemicals and dosage forms

Pharmaceutical grade moxifloxacin (MOXF) base was a kind gift from Bayer (Riyadh, KSA), its purity was 100.2 ± 0.65. Acetaldehyde (ACD; Sigma Chemical Co., St. Louis, USA) was 70% (v/v), prepared in methanol.

2,3,5,6-Tetrachloro-1,4-benzoquinone (TCBQ; Sigma Chemical Co., USA) was 2×10^{-2} mol l^{-1}, freshly prepared in dioxane. All solvents and other chemicals used through this study were of analytical grade.

Avalox® tablets (Bayer AG.Germany/Allemagne) are labeled to contain 400 mg MOXF per tablet (batch number, BXFOCJI).

2.3. Preparation of standard and sample solutions

2.3.1. Preparation of stock standard solution

Into a 50-ml calibrated flask, an accurately weighed amount (100 mg) of MOXF was dissolved in 40 ml of methanol. For complete dissolution of MOXF, the solution was sonicated for 5 min. The resulting solution was completed to volume with the same solvent. This stock solution (2 mg ml^{-1}) was diluted with butanol to obtain working concentrations in the range of 50–1500 g ml^{-1}.

2.3.2. Preparation of dosage forms sample solution

Twenty tablets were weighed and finely powdered. A quantity of the mixed powder equivalent to 100 mg of MOXF was transferred into a 50-ml calibrated flask, dissolved in 25 ml of methanol, swirled and sonicated for 5 min, completed to volume with the same solvent, shaken well for 10 min, and filtered. The first portion of the filtrate was rejected, and 25 ml of the filtrate was diluted with butanol to obtain working concentrations in the range of 50–1500 μg ml^{-1}.

2.4. General analytical procedures and data treatment

One milliliter of the standard or sample solution (50–1500 μg ml^{-1}) was transferred into 10-ml calibrated flasks. One milliliter of the ACD solution (70%, v/v in methanol)

and 1 ml of TCBQ (2×10^{-2} M in dioxane) were added. The reaction mixture was mixed and completed to volume with butanol. After dilution and mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded (at 652 nm) as a function of time against reagent blank treated similarly.

The kinetic data that has been recorded were transformed to the Slide Write Plus software, version 5.011 (Advanced Graphics Software, Inc., CA, USA) for curve fitting, regression analysis, and statistical calculations. The initial rate (K) of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration curve was constructed by plotting the logarithm of the initial rate (log K) of reaction versus logarithm of the concentration (log C) of MOXF. Alternatively, the calibration curve was constructed by plotting the absorbance measured after a fixed time of 5 min.

2.5. Determination of molar ratio of the reactions

2.5.1. For MOXI with ACD

The limiting logarithmic method (Rose, 1964) was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments were carried using increasing ACD concentrations (3×10^{-2}–1.8×10^{-1} M) at a fixed MOXF concentration (1.32×10^{-4} M). The second set of experiments were carried using increasing MOXF concentrations (2.54×10^{-5}–2.54×10^{-4} M) at fixed ACD concentration (0.2 M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the ACD and MOXF concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

2.5.2. For MOXI with TCBQ

The limiting logarithmic method (Rose, 1964) was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments were carried using increasing TCBQ concentrations (1.3×10^{-4}–2.0×10^{-3} M) at a fixed MOXF concentration (1.32×10^{-4} M). The second set of experiments were carried using increasing MOXF concentrations (2.54×10^{-5}–2.54×10^{-4} M) at fixed TCBQ concentration (2.0×10^{-3} M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the TCBQ and MOXF concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

3. Results and discussion

3.1. Involved reaction and absorption spectra

The reaction involved in the present study was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of MOXF by its reaction with TCBQ in presence of ACD. The formation of this colored product was monitored spectrophotometrically at its maximum absorption peak (652 nm). The absorption spectrum for the reaction product is given in Fig. 1. The following sections describe the optimization of different factors affecting the reaction, kinetics, and the use of the optimized conditions in the development of the assay procedures.

3.2. Optimization of reaction conditions

The factors affecting reaction conditions (concentrations of ACD and TCBQ reagents, temperature, and the diluting solvent) were studied by altering each variable in turn while keeping the others constant. The intensity of the developed color was recorded as a function of the concentrations of ACD and TCBQ reagent. It was found that the color intensity was dependent on the concentration of both reagents (Fig. 2). The highest color intensity was attained when the concentrations of ACD and TCBQ in the final reaction solution were 7% (v/v) and 2×10^{-3} M, respectively; these concentrations were used in all the subsequent experiments. The reaction was carried out at room temperature (25 ± 5 °C) and at elevated temperatures. It was found that the color intensity decreased significantly when the reaction temperature increased (Fig. 3). This was probably attributed to the instability of the colored product at elevated temperature. Therefore, the further experiments were carried out at room temperature.

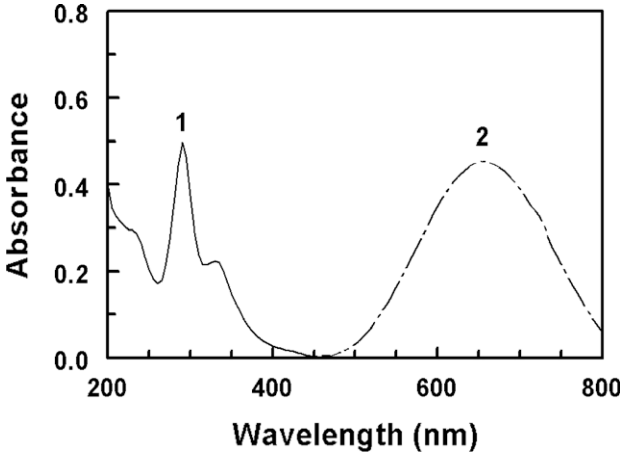

Figure 1 Absorption spectra of 6 μg ml^{-1} of MOXF (1) and the reaction product (2) of MOXF (50 μg ml^{-1}) with TCBQ (2×10^{-3}) in presence of ACD (7%, v/v).

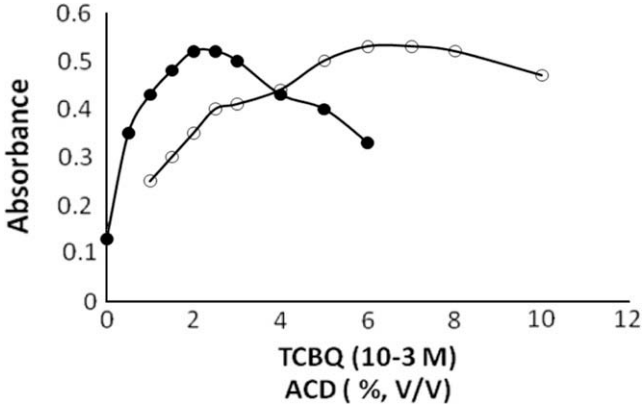

Figure 2 Effect of ACD (○) and TCBQ (●) concentrations on the absorption intensity of their reaction with MOXF (50 μg ml^{-1}).

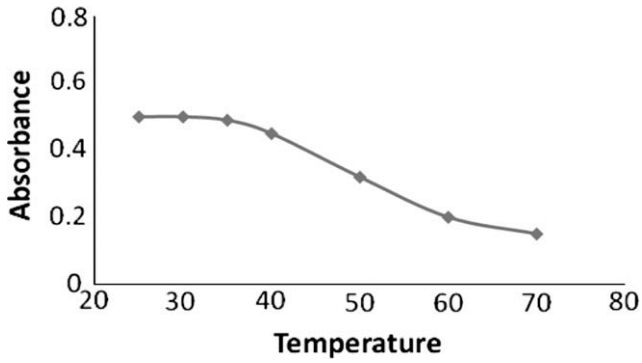

Figure 3 Effect of temperature on the reaction of MOXF (50 μg ml^{-1}) with TCBQ (2×10^{-3}) in presence of ACD (7%, v/v).

In order to select the most appropriate solvent for dilution, different solvents were tested: methanol, ethanol, propan-2-ol, butanol, acetonitrile, acetone, and dimethylformamide. The highest color intensity was attained when butanol was used as a diluting solvent; therefore it was selected for the further investigations.

3.3. Stoichiometry, mechanism, and kinetics of the reaction

The stoichiometry of the reaction between MOXF and each of ACD and TCBQ was investigated by limiting logarithmic method (Rose, 1964). The time-dependence of the reaction between MOXF and ACD was first investigated, and it was found that the reaction proceeds instantaneously (data not shown). Upon studying the stoichiometric ratio of MOXF:ACD using a fixed concentration of MOXF (1.32×10^{-4} M), two straight lines with comparable slopes were obtained indicating that 1:1 ratio was involved in the reaction of MOXF with ACD (Fig. 4). Upon studying the ratio of MOXF:TCBQ, the slope of the line that was generated using varying [MOXF] with fixed equimolar concentrations of ACD and TCBQ was ~2-folds of the slopes of the other lines. This indicated that the ratio of MOXF:TCBQ was 2:1 which was confirmed also by Job's method (Job, 1936). Based on these ratios, the reaction pathway was postulated to proceed as shown in Fig. 5. MOXF, via its piprazinyl NH, reacted with ACD and produced the

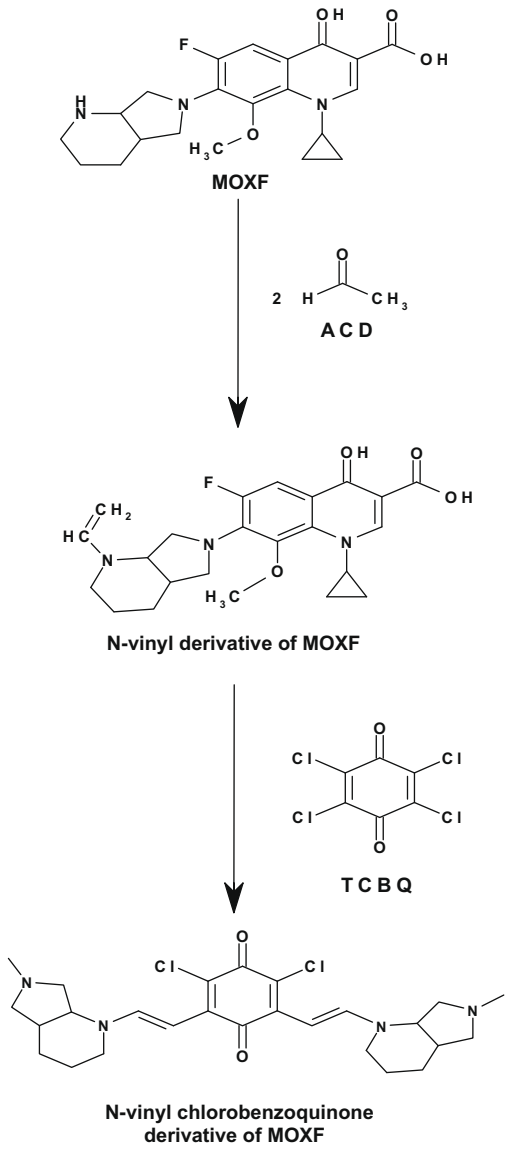

Figure 5 Scheme for the reaction pathway of MOXF with ACD and TCBQ.

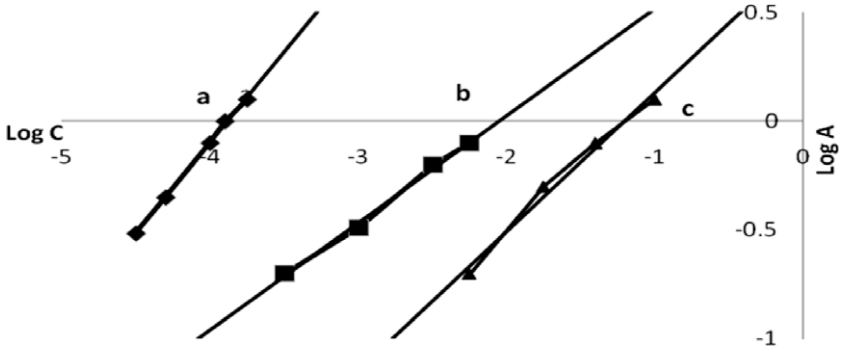

Figure 4 Limiting logarithmic plots for molar reactivity of MOXF with ACD and TCBQ. log *C* and log *A* are the logarithm of the concentration and absorbance, respectively. The first line (a): log *A* versus log [MOXF]; the second line (b): log *A* versus log [TCBQ]; the third line (c): log *A* versus [ACD].

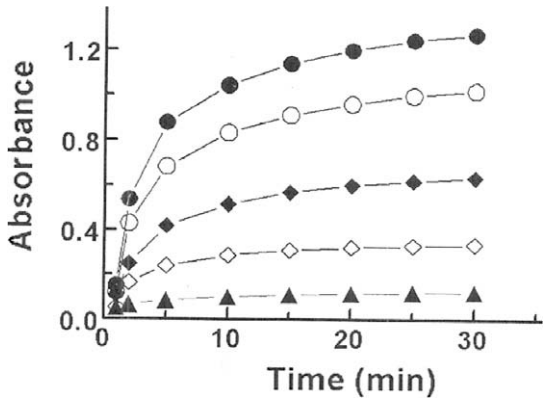

Figure 6 Absorbance–time curves for the reaction of varying concentrations of MOXF (-▲-, 0.32), (-◇-, 0.75), (-◆-, 1.50), (-○-, 2.00), (-●-, 2.54) ($M \times 10^{-4}$) (with TCBQ (2×10^{-3} M) in presence of ACD (7%, v/v).

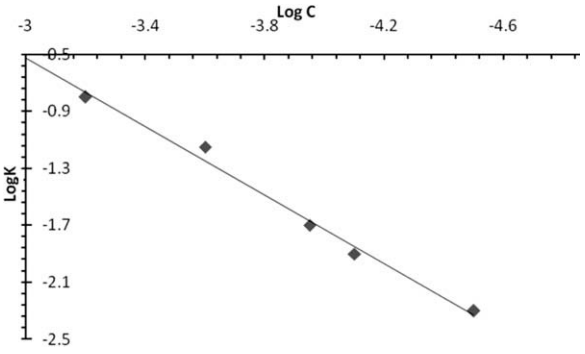

Figure 7 Linear plot for $\log C$ versus $\log K$ for the kinetic reaction of MOXF with TCBQ (2×10^{-3} M) in presence of ACD (7%, v/v) and C is [MOXF], (0.25×10^{-4}–2.54×10^{-4} M); K is the reaction rate (s^{-1}).

N-vinyl derivative of MOXF, which was subsequently coupled with TCBQ and produced the colored N-vinyl chlorobenzoquinone derivative of MOXF.

Under the optimum conditions, the absorbance–time curves for the reaction of varying MOXF concentrations (0.32×10^{-4}–2.54×10^{-4} M) with a fixed concentration of TCBQ (2×10^{-3} M) in presence of ACD (7%, v/v) were generated (Fig. 6). The initial reaction rates (K) were determined from the slopes of these curves. The logarithms of the reaction rate ($\log K$) were plotted as a function of logarithms of MOXF concentrations ($\log C$) (Fig. 7). The regression analysis for the values was performed by fitting the data to the following equation:

$$\log K = \log k' + n \log C$$

where K is reaction rate, k' is the rate constant, C is the molar concentration of MOXF, and n (slope of the regression line) is the order of the reaction. A straight line with slope values of 1.164 (≈1) was obtained confirming that the reaction was first order. However under the optimized reaction conditions, the concentrations of ACD and TCBQ were in much more excess than that of MOXF in the reaction solution. Therefore, the reaction was regarded as a pseudo-first order reaction. Since the reaction between MOXF and ACD was instantaneous, the rate- limiting step in the overall reaction was the condensation of the N-vinyl-MOXF with TCBQ reagent.

3.4. The apparent rate constant and activation energy

The absorbance–time curves at different temperatures (20–40 °C) were generated using fixed concentration of MOXF (1.0×10^{-4} mol l^{-1}), ACD (7%, v/v), and TCBQ (2×10^{-3} mol l^{-1}). From these curves the apparent rate constants were calculated. The activation energy, defined as the minimum kinetic energy that a molecule possess in order to undergo a reaction, was determined using Arrhenius equation (Martin et al., 1983):

$$\log k = \log F - E_a/2.303RT$$

where k is the apparent rate constant, F is the frequency factor, E_a is the activation energy, T is the absolute temperature (°C + 273), and R is the gas constant (1.987 calories deg^{-1} mol^{-1}). The values of $\log k$ were plotted as a function of $1/T$. Straight line with slope value of -0.4444 ($= -E_a/2.303R$) was obtained. From these data, the activation energy was calculated and found to be 6.65 kJ mol^{-1}. This low activation energy explained that the proposed reaction could be easily takes place under mild conditions, and ACD–TCBQ combination could be used as useful analytical reagents in the spectrophotometric determination of MOXF.

3.5. Quantitation methods

3.5.1. Initial rate method

The initial rate of the reaction for MOXF followed a pseudo-first order and were found to obey the following equation:

$$K = \Delta A/\Delta t = K^{\backslash} C^n$$

where K is the reaction rate, A is the absorbance, t is the measuring time, $K^{\backslash}$ is the pseudo-first order rate constant, C is the molar concentration of MOXF and n is the order of the reaction. The logarithmic form of the above equation is written as follows:

$$\log K = \log \Delta A/\Delta t = \log K^{\backslash} + n \log C$$

Regression analysis, using the method of least square was performed for the data. The value of n (slope) was 1.16 (≈1) in the regression equation, confirmed that the reaction was first order with respect to MOXF concentration, the relation is linear in the range of 5–100 μg ml^{-1}, with good correlation coefficient (0.9992). The limit of detection (LOD) and limit of quantification (LOQ) were calculated and found to be 2.0 and 6.0 μg ml^{-1}, respectively. These low values confirmed the high sensitivity of the method and consequently its capability to determine low amounts of MOXF.

3.5.2. Fixed time method

In this method, the absorbance of the reaction solution containing varying amounts of MOXF was measured at a pre-selected fixed time. Calibration plots of absorbance versus the concentrations of MOXF were established at fixed periods of time for the reaction. The regression equations, coefficients of correlation, and limits of detection are given in Table 1. Obviously, the LOD values decreased as the measuring fixed time increased. The widest linear ranges were obtained at 2

Table 1 Analytical parameters for the proposed fixed time spectrophotometric method for determination of MOXF.

Reaction time (min)	Linear range (μg ml^{-1})	Intercept	Standard deviation of intercept	Slope	Standard deviation of slope	Correlation coefficient	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
2	20–200	0.0211	0.0134	0.0049	0.000362	0.9967	7.0	21.0
5	15–150	0.0038	0.0061	0.0077	0.000205	0.9996	5.0	15.0
10	10–130	0.0041	0.0093	0.0087	0.000331	0.9995	2.8	8.5
15	8–115	0.0035	0.0094	0.0108	0.000190	0.9995	2.5	7.6
20	6–100	0.0029	0.0065	0.0115	0.000221	0.9997	2.5	6.8
25	6–75	0.0006	0.0081	0.0120	0.000409	0.9996	2.0	6.0
30	6–80	0.0002	0.0055	0.0125	0.000158	0.9997	1.5	4.8

Table 2 Accuracy and precision for the initial rate and fixed-time spectrophotometric methods for determination of MOXF.

Concentration (μg ml^{-1})	Recovery (± RSD)[a]			
	Initial rate method		Fixed time method	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay
20	100.2 ± 1.87	100.6 ± 1.68	99.9 ± 0.98	99.3 ± 0.15
50	101.6 ± 1.04	98.9 ± 1.43	99.7 ± 0.38	100.2 ± 0.08
90	100.1 ± 0.61	99.5 ± 0.82	100.0 ± 0.15	100.0 ± 0.02

[a] Values are the mean of at least three determinations.

Table 3 Results of analysis of MOXF tablets by the proposed methods and comparison method.[a]

Dosage form	Proposed method		Comparison method[a]
	Initial rate method	Fixed time method	
Avalox® tablets, 400 mg MOXF/tablet			
Label claim[*] (% ±RSD)	101.25 ± 0.73	100.92 ± 0.65	101.86 ± 0.53
N	4	4	4
Variance	0.53	0.42	0.28
F-test	1.89	1.40	(9.28)[**]
t-test	1.36	2.23	(2.36)[**]

[a] Motwani et al. (2007).
[*] Values are mean ± RSD of four determinations.
[**] Values in parenthesis are tabulated values at $p = (0.05)$.

and 5 min, however poor linearity was obtained at 2 min, as the correlation coefficient was low. According to the ICH guidelines for validation of analytical procedures (International Conference on Harmonization, 1995), the LOD is not required to be part of the validation. Therefore, on the basis of wider concentration range and less time of analysis, the fixed time of 5 min was recommended for analytical procedure.

3.6. Validation of the proposed methods

3.6.1. Accuracy and precision

The accuracy and precision of the proposed kinetic spectrophotometric method were determined at three concentration levels (20, 50, and 90 μg ml^{-1}) of MOXF by analyzing five replicate samples of each concentration by both the initial rate and fixed time methods. The relative standard deviations (RSD) for the results did not exceed 2% (Table 2), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of MOXF in its pharmaceutical tablets.

3.6.2. Application of the proposed methods

It is evident from the results obtained previously that the proposed initial rate and fixed time methods of the proposed kinetic spectrophotometric method for determination of MOXF gave satisfactory results with the analysis of MOXF in bulk. The methods were applied on the analysis of commercial MOXF tablets dosage form. The concentration of MOXF was computed from its corresponding regression equations. The results of the proposed methods (initial rate or fixed time) were statistically compared with those of the reported method (Motwani et al., 2007), in respect to the accuracy and precision. The obtained mean recoveries and relative standard deviations of the labeled amounts were 101.25 ± 0.73% and 100.9 ± 0.65% for the initial rate and the fixed time methods, respectively (Table 3). In the *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at

Table 4 The analytical figures of merit of the previously-mentioned spectrophotometric methods.

Method/reagent	Reaction conditions	λ_{max} (nm)	Beer's range (μg ml^{-1})	LOD (μg ml^{-1})	Ref.
Proposed initial rate	25 °C/10 min	655	5–100	2.0	This work
Proposed fixed time	25 °C/5 min	655	15–150	5.0	This work
UV-spectrophotometry					
In 0.1 N hydrochloric acid	pH 1.2	296	1–12	0.0402	Motwani et al. (2007)
In phosphate buffer	pH 7.4	289	1–14	0.1217	Motwani et al. (2007)
Formation of ion-pair associates					
Ammonium reineckate*	5×10^{-3} M ammonium reineckate + 0.01 M HCl	525	100–1100	45	Al-Ghannam (2008)

* The ion pair associate, as precipitate, was dissolved in acetone before measuring.

95% confidence level. This indicated similar precision and accuracy in the analysis of MOXF in its dosage forms. It is worth noting that all the proposed kinetic spectrophotometric methods were performed in the visible region away from the UV-absorption region of the UV-absorbing interfering excipient materials that might be co-extracted from the MOXF-containing tablets.

3.7. Comparison of the proposed methods with the reported spectrophotometric methods

The proposed methods, because they involve measurements in visible region, are more selective than the previously reported spectrophotometric method that involved measurements in the ultraviolet region (Motwani et al., 2007; Venugopal and Saha, 2005). As well, the proposed methods are superior to the reported methods that were based on the ion-pair associates (Al-Ghannam, 2008), as the proposed methods do not require elaborate treatment of the samples, careful adjustment of the critical optimum pH of the reaction medium, and/or tedious liquid-liquid extraction for the chromophores. Furthermore, the proposed methods have wider linear dynamic ranges than that of many of the reported methods (Table 4). These advantages encourage the application of the proposed methods in routine analysis of MOXF in quality control laboratories, as alternatives for the existing methods.

4. Conclusion

The present study described, for the first time, a selective and simple kinetic spectrophotometric method for the determination of MOXF in its tablet. The proposed initial rate and fixed time methods can be easily applied as they do not require elaborate treatment of the samples and/or tedious procedures for extraction of the chromophore. As well, both methods are sensitive enough for analysis of lower amounts of MOXF. Furthermore, the proposed methods do not require expensive instruments and/or critical analytical reagents. These advantages give the proposed methods a great value and encourage its application to the analysis of MOXF in quality control laboratories.

References

Al-Ghannam, S.M., 2008. Spectrochim. Acta A 69, 1188.
Balfour, J.A.B., Lamb, H.M., 2000. Drugs 59, 115.
Biedenbach, D.J., Barrett, M.S., Croco, M.A.T., Jones, R.N., 1998. Diagn. Microbiol. Infect. Dis. 32, 45.
Chamjangali, M.A., Keley, V., Bagherian, G., 2006. Anal. Sci. 22, 333.
Chan, K.P., Chu, K.O., Lai, W.W.K., Choy, K.W., Wang, C.C., Lam, D.S.C., Pang, C.P., 2006. Anal. Biochem. 353, 30.
Darwish, I.A., 2005. Anal. Chim. Acta 551, 222.
Darwish, I.A., Hussein, S.A., Mahmoud, A.M., Hassan, A.I., 2008. Spectrochim. Acta A 69, 33.
Darwish, I.A., Abdine, H.H., Amer, S.M., Al-Rayes, L.I., 2009. Spectrochim. Acta A 72, 897.
Faria, A.F., de Souza, M.V.N., de Almeida, M.V., de Oliveira, M.A.L., 2006. Anal. Chim. Acta 579, 185.
Ilango, K., Valetina, P., Lakshmi, K.S., Arvind, C., Rachel, A.S., Bhaskar, R.V., Kiran, K.A., 2006. Indian J. Pharm. Sci. 68, 273.
International Conference on Harmonization, 1995. ICH harmonised tripartite guidline-text on validation of analytical procedures. Fed. Regist. 60, 11260.
Jane, J., Subrahmanyam, E.V.S., Sathyanarayana, D., 2006. Asian J. Chem. 18, 3210.
Job, P., 1936. In: Advanced Physicochemical Experiments, second ed. Oliner and Boyd, Edinburgh, 1964, p. 54. Ann. Chem. 16, 97.
Kumar, A.K.H., Ramachandran, G., in press. J. Chromatogr. B.
Martin, A., Swarbrick, J., Cammarata, A., Chun, A., 1983. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Science, third ed. Lea & Febiger, Philadelphia, p. 371.
Motwani, S.K., Chopra, S., Ahmad, F.J., Khar, R., 2007. Spectrochim. Acta A 68, 250.
Muijsers, R.B.R., Jarvis, B., 2002. Drugs 62, 967.
Ocana, J.A., Barragan, F.J., Collejon, M., 2000. Analyst 125, 2322.
Patel, P.V., Suhagia, B.N., Patel, C.N., Patel, M.M., Patel, G.C., Patel, G.M., 2005. Indian J. Pharm. Sci. 67, 356.
Rahman, N., Anwar, N., Kashif, M., 2006. Chem. Pharm. Bull. 54, 33.
Rose, J., 1964. Advanced Physicochemical Experiments. Pitman, London, p. 67.
Trindade, M.A.G., da Silva, G.M., Ferreira, V.S., 2005. Microchem. J. 81, 209.
Uiu, S.T., 2007. J. Pharm. Biomed. Anal. 43, 320.
Venugopal, K., Saha, R.N., 2005. Farmaco 60, 906.
Vishwanathan, K., Bartlett, M.G., Stewart, J.T., 2002. J. Pharm. Biomed. Anal. 30, 961.
Wise, R., Andrews, J.M., Marshall, G., Hartman, G., 1999. Antimicrob. Agents Chemother. 43, 1508.