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A Comparative Study of Different Approaches for Stability-Indicating Determination of Moxifloxacin Hydrochloride in Presence of Its Acidic-Induced Degradation Product

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ABSTRACT

This work describes five simple, specific and accurate spectrophotometric methods which developed and validated for determination of moxifloxacin hydrochloride in presence of its acidic degradation product without previous separation, namely; bivariate, graphical absorbance ratio, area under the curve, simultaneous equation and dual wavelength. Calibration graphs were established in the range of 1-9 µg/ml with good correlation coefficients. The developed methods have been successfully applied for the simultaneous analysis of moxifloxacin hydrochloride in its pharmaceutical dosage form. The methods were validated as per ICH guidelines; accuracy, precision and repeatability were found to be within the acceptable limit.

1. INTRODUCTION

Moxifloxacin hydrochloride is 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid hydrochloride, figure (1). It is a member of the fluoroquinolone antibiotics enter bacteria through porin channels and exhibit antimicrobial effects on DNA gyrase (bacterial topoisomerase II) and bacterial topoisomerase IV. Inhibition of DNA gyrase results in relaxation of supercoiled DNA, promoting DNA strand breakage. Inhibition of topoisomerase IV impacts chromosomal stabilization during cell division, thus interfering with the separation of newly replicated DNA. It is a light yellow powder or crystals, slightly hygroscopic. Sparingly soluble in water; slightly soluble in alcohol; practically insoluble in acetone [1]. The literature is enriched with several techniques for determination of moxifloxacin hydrochloride in pharmaceutical dosage forms and/or biological fluids, including HPLC methods [2-10], UPLC [11, 12], capillary electrophoresis [13, 14], TLC densitometry [15-19], electrochemical methods [20,25], spectrofluorometry [26-30], atomic absorption spectrometric methods [31,32] visible spectrophotometric methods [33-39] and UV spectrometric methods [40-44].

Reviewing the literature on the determination of moxifloxacin hydrochloride revealed the lack of any stability indicating spectrophotometric methods for the determination of moxifloxacin hydrochloride in presence of its acid degradation product. The aim of this work is to develop a simple, economic, rapid, sensitive, accurate and precise stability indicating methods for determination of moxifloxacin hydrochloride in presence of its acid degradation product without sophisticated instruments or any separation steps.

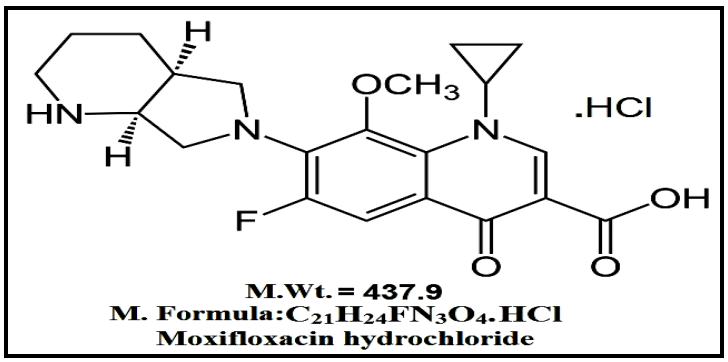

Fig. (1): Structural formula of moxifloxacin hydrochloride.

2. Theory:

2.1. Theory of bivariate method [45]:

The principle of bivariate calibration is the measurement of two components (X and Y) at two selected wavelengths (λ_1 and λ_2) to obtain two equations:

$$A_{XY1} = m_{X1}C_X + m_{Y1}C_Y + e_{XY1} \quad \textbf{(1)}$$

$$A_{XY2} = m_{X2}C_X + m_{Y2}C_Y + e_{XY2} \quad \textbf{(2)}$$

- The resolution of such equations set allows the evaluation of C_x and C_y values:

$$C_X = (A_{XY1} - e_{XY1} - m_{Y1}C_Y) / m_{X1} \quad \textbf{(3)}$$

$$C_Y = [m_{X2} (A_{XY1} - e_{XY1}) + m_{X1} (e_{XY2} - A_{XY2})] / m_{X2}m_{Y1} - m_{X1}m_{Y2} \quad \textbf{(4)}$$

Where:

- C_X, C_Y are the concentration of component X, component Y.

- m_{X1}, m_{X2} are the slope values of component X at λ_1, λ_2.

- m_{Y1}, m_{Y2} are the slope values of component Y at λ_1, λ_2.

- A_{XY1}, A_{XY2} are the absorbance values of the binary mixture at λ_1, λ_2.

- e_{XY1}, e_{XY2} are the sum of the intercepts of components X, Y at λ_1, λ_2.

- According to Kaiser method, the slope values of the linear regression equations for both components at different wavelengths were used to calculate the sensitivity matrices (K**)** to find out the optimum pair of wavelengths (highest matrix value) at which the binary mixture was determined.

$$K = \begin{vmatrix} m_{X1} & m_{Y1} \\ m_{X2} & m_{Y2} \end{vmatrix} \quad \textbf{(5)}$$

2.2. Theory of graphical absorbance ratio method [46,47]:

According to graphical absorbance method, uses the ratio of absorption at two selected wavelengths, one is at λ_{iso} and other being the λ_{max} of one of the two components. Two equations were constructed as described below, using the relationship $a_{x1}=a_{y1}$ and L=1. Equations are;

$$At\ \lambda_1 : A_1 = a_{x1}C_x + a_{y1}C_y \quad (because\ ax_1 = ay_1) \qquad (1)$$

$$At\ \lambda_2 : A_2 = a_{x2}C_x + a_{y2}C_y \qquad (2)$$

Dividing equation (2) by (1), we get;

$$A_2/A_1 = (a_{x2}C_x + a_{y2}C_y)/(a_{x1}C_x + a_{y1}C_y) \qquad (3)$$

Let $C_x/(C_x + C_y) = F_x$&$C_y/(C_x + C_y) = F_y$

Dividing equation (3) by $\mathbf{C_x+C_y}$, we get;

$$A_2/A_1 = (a_{x2}F_x + a_{y2}F_y)/(a_{x1}F_x + a_{y1}F_y)$$

,But $F_y = 1 - F_x$

$$A_2/A_1 = (a_{x2}F_x + a_{y2} - a_{y2}F_x)/a_{x1} \qquad (4)$$

,Because $a_{x1} = a_{y1}$

$$A_2/A_1 = (a_{x2}F_x/a_{x1}) - (a_{y2}F_x/a_{y1}) + (a_{y2}/a_{y1})$$

Let $a_{x2}/a_{x1} = Q_x$&$a_{y2}/a_{y1} = Q_y$&$A_2/A_1 = Q_M$

So, $Q_M = F_x Q_x - F_x Q_y + Q_y$

$$F_x = (Q_M - Q_y)/(Q_x - Q_y) \qquad (5)$$

This equation gives the fraction of mixture that determines the absolute concentration of X and Y.

$$C_x/(C_x + C_y) = (A_2/A_1) - (a_{y2}/a_{y1})/(a_{x2}/a_{x1}) - (a_{y2}/a_{y1}) \qquad (6)$$

Both equation (5) & (6) gives the fraction, rather than the concentration of X and consequently of Y in the mixture in the term of absolute ratio. As these are independent of concentration only approximate rather than accurate.

If the absolute concentration of X & Y than rearrange equation (1), we get;

$$C_x + C_y = A_1/a_{x1} \qquad (7)$$

From equations (6) & (7), we get;

$$C_x/(A_1/a_{x1}) = (Q_M - Q_y)/(Q_x - Q_y)$$

$$C_x = \{(Q_M - Q_y)/(Q_x - Q_y)\} \times (A_1/a_{x1}) \qquad (8)$$

$$\&C_y = \{(Q_M - Q_x)/(Q_y - Q_x)\} \times (A_1/a_{y1}) \qquad (9)$$

Finally, equations (8 & 9) gives the absolute concentration value of component X & Y.

2.3. Theory of area under the curve method [48]:

Consider a binary mixture consisting of two components X and Y, from the spectra of two components, following information is obtained:

- $AUC^{X}_{\lambda 1-\lambda 2}$: area under curve for component X at the wavelength range λ_1 - λ_2.

- $AUC^{X}_{\lambda 3-\lambda 4}$: area under curve for component X at the wavelength range λ_3 - λ_4.
- $AUC^{Y}_{\lambda 1-\lambda 2}$: area under curve for component Y at the wavelength range λ_1 - λ_2.
- $AUC^{Y}_{\lambda 3-\lambda 4}$: area under curve for component Y at the wavelength range λ_3 - λ_4.

The total area under the curve of a mixture at a particular wavelength range is equal to the sum of area under curve of the individual components at same wavelength range. The area under curve of the mixture containing component X and Y can be given as follows:

$$AUC_{\lambda 1-\lambda 2} = AUC^{X}_{\lambda 1-\lambda 2} + AUC^{Y}_{\lambda 1-\lambda 2} \quad (1)$$

$$AUC_{\lambda 3-\lambda 4} = AUC^{X}_{\lambda 3-\lambda 4} + AUC^{Y}_{\lambda 3-\lambda 4} \quad (2)$$

Now the above equations can also be written as follows:

$$AUC_{\lambda 1-\lambda 2} = A^{X}_{\lambda 1-\lambda 2}\, bC^{X} + A^{Y}_{\lambda 1-\lambda 2}\, bC^{Y} \quad (3)$$

$$AUC_{\lambda 3-\lambda 4} = A^{X}_{\lambda 3-\lambda 4}\, bC^{X} + A^{Y}_{\lambda 3-\lambda 4}\, bC^{Y} \quad (4)$$

Where,

$$A_{\lambda 1-\lambda 2} = AUC_{\lambda 1-\lambda 2} / Conc.in\ \mu g/ml$$

$$A_{\lambda 3-\lambda 4} = AUC_{\lambda 3-\lambda 4} / Conc.in\ \mu g/ml$$

By applying "Cramer's Rule'' and "Matrix Method'', the concentration of component X and component Y can be determined as follows:

$$C^{X} = \frac{(A^{Y}_{\lambda 1-\lambda 2}\ AUC_{\lambda 3-\lambda 4}) - (A^{Y}_{\lambda 3-\lambda 4}\ AUC_{\lambda 1-\lambda 2})}{(A^{Y}_{\lambda 1-\lambda 2}\ A^{X}_{\lambda 3-\lambda 4}) - (A^{Y}_{\lambda 3-\lambda 4}\ A^{X}_{\lambda 1-\lambda 2})} \quad (5)$$

$$C^{Y} = \frac{(A^{X}_{\lambda 1-\lambda 2}\ AUC_{\lambda 3-\lambda 4}) - (A^{X}_{\lambda 3-\lambda 4}\ AUC_{\lambda 1-\lambda 2})}{(A^{Y}_{\lambda 1-\lambda 2}\ A^{X}_{\lambda 3-\lambda 4}) - (A^{Y}_{\lambda 3-\lambda 4}\ A^{X}_{\lambda 1-\lambda 2})} \quad (6)$$

2.4. Theory of simultaneous equation method [48]:

Consider a multicomponent system consisting of two components X and Y, each of which absorbs at the λ_{max} of the other.λ_1 being the wavelength of maximum absorbance of **X** and λ_2 being the wavelength of maximum absorbance of **Y**, it may be possible to determine both components by the technique of simultaneous equation method using "Cramer's Rule'' and "Matrix Method''.

- **The information required is:**

➢ The absorptivity's of **X** at λ_1 and λ_2, a_{X1} and a_{X2} respectively.

➢ The absorptivity's of **Y** at $\lambda 1$ and $\lambda 2$, a_{Y1} and a_{Y2} respectively.

➢ The absorbance of the diluted sample at $\lambda_1$and λ_2, A1 and A2 respectively.

➢ C_X and C_y be the concentrations of **X** and **Y** respectively in the diluted sample.

Thus the absorbance of the mixture at λ_1 and λ_2 may be expressed as follows:

At λ_1 $$A_1 = [a_{X1}\, bC_X] + [a_{Y1}\, bC_Y] \quad \textbf{(1)}$$

At λ_2 $$A_2 = [a_{X2}\, bC_X] + [a_{Y2}\, bC_Y] \quad \textbf{(2)}$$

For measurements in 1 cm cell, b = 1, rearrange eq. **(2)**:

$$C_Y = \frac{A_2 - a_{X2}C_X}{a_{Y2}}$$

Substituting for C_Y in eq. (1) and rearranging gives

$$C_X = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad \textbf{(3)}$$

$$C_Y = \frac{A_1 a_{X2} - A_2 a_{X1}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad \textbf{(4)}$$

Using the above two equations (3, 4) the concentration of component X and component Y in the sample mixture can be determined.

2.5. Theory of dual wavelength method [49]:

If we have a mixture of two component (X and Y), for determination of compound X without interference from Y we select two wavelengths at which $A_{X1} \neq A_{X2}$ but $A_{Y1} = A_{Y2}$

Where A_{X1} and A_{X2} are the absorbances of compound X at λ_1 and λ_2 respectively and A_{Y1} and A_{Y2} are the absorbance of compound Y at λ_1 and λ_2 respectively.

For the mixture of X and Y

At λ_1 $A_{M1} = A_{X1} + A_{Y1}$ **(1)**

At λ_2 $A_{M2} = A_{X2} + A_{Y2}$ **(2)**

Where A_{M1} and A_{M2} are the absorbances of mixture at λ_1 and λ_2 respectively.

By taking the absorbance difference between λ_1 and λ_2:

$A_{M1} - A_{M2} = A_{X1} + A_{Y1} - A_{X2} - A_{Y2}$ but $(A_{Y1} - A_{Y2})$ equal zero

So $A_{M1} - A_{M2} = A_{X1} - A_{X2}$ **(3)**

From equation (3) we note that the absorbance difference is related to compound X without interference from compound Y.

3. MATERIALS

3.1. Instruments:

- Shimadzu UV-Visible 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells.
- Hot plate (Torrey pines Scientific, USA).
- Rotary evaporator (Scilogex-RE 100-pro, USA)
- pH meter Jenway 3510 (England) with Ag/AgCl reference electrode no 924017-LO3-Q11C.

3.2. Materials:

- Pure moxifloxacin hydrochloride (99.45%) was kindly provided by EVA Pharmaceutical Industrial Company, Cairo, Egypt.
- Moxiflox® tablet: labeled to contain 400 mg of moxifloxacin hydrochloride per tablet, manufactured by EVA Pharmaceutical Industrial Company; (batch number 505954), purchased from local market.
- Hydrochloric acid, Sodium hydroxide and Methanol (El-Nasr Co., Egypt).

3.3. Standard solutions:

A standard solution of moxifloxacin hydrochloride (100µg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of methanol and complete to 100 ml with methanol.

3.4. Preparation of acidic-induced degradation product [40]:

100 mg of pure moxifloxacin hydrochloride powder was treated with 25 ml 2M HCl in a 100-ml round bottomed flask, the solution was heated under reflux for 10 hours. After cooling the solution was to pH = 7-8 using 2M KOH, evaporated to dryness under vacuum. The obtained residue was extracted three times with 25 ml methanol, filtered into 100-ml volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradate derived from 1 mg/ml of moxifloxacin hydrochloride. Working solution of degradate (100 µg/ml) was obtained by dilution of the stock solution with methanol.

3.5. Spectral characteristics:

The absorption spectra of moxifloxacin hydrochloride and its degradation product were recorded over the range 200-400 with linearity range of (1 – 9) µg/ml. The zero-order absorption spectra of moxifloxacin hydrochloride and its degradate show severe overlap, which does not permit direct determination of moxifloxacin hydrochloride in presence of its degradate.

3.6. Laboratory prepared mixtures:

Aliquots of moxifloxacin hydrochloride solution (100 µg/ml) containing (70–10 µg) were transferred and mixed with aliquots of its degradate solution (100 µg/ml) containing (20–80 µg)

in a set of 10-ml volumetric flaks. The volumes were completed to mark with methanol and mixed well.

3.7. Pharmaceutical formulation:

Ten Moxiflox® tablets (400 mg/tablet) were weighed and finely powdered. An accurately weighed portion equivalent to 10 mg of moxifloxacin hydrochloride was extracted three times with 25 ml methanol, filtered into 100 ml volumetric flask then the volume was adjusted with methanol to obtain a concentration of 100 µg/ml. Pharmaceutical solution was diluted to the working calibration ranges.

4. METHODS

4.1. Construction of calibration curves (linearity):

Different aliquots equivalent to (10–90) µg of moxifloxacin hydrochloride and (10–90) µg of its degradate were accurately transferred from their standard solutions (100 µg/ ml) into two separate series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank.

4.1.1. Bivariate method:

The absorbance of the drug and its degradate were measured at 275 and 290 nm versus the concentrations in µg/ml and then the corresponding regression equations were computed at the selected wavelengths for both moxifloxacin hydrochloride and its degradate. Moxifloxacin hydrochloride concentrations were calculated using equations (3&4) mentioned under section "2.1".

4.1.2. Graphical absorbance ratio method:

The absorbance values at 281.2 nm (iso-absorptive point) and at 291 nm (λ_{max}) versus the concentrations in µg/ml were plotted to get the calibration graph and the regression equations. Moxifloxacin hydrochloride concentrations were calculated using equation (8) mentioned under section "2.2".

4.1.3. Area under the curve method:

The area under the curves obtained from the scanned spectra over the ranges of wavelengths (275 - 280) and (289 - 294) nm were calculated versus the concentrations in µg/ml and then the corresponding regression equations were computed for both moxifloxacin hydrochloride and its degradate. The constant 'A' values were determined as, A= area under curve of component (from 275 to 280 nm or 289 to 294 nm)/concentration of the component. The intact concentrations were calculated using equation (5) mentioned under section "2.3".

4.1.4. Simultaneous equation method:

The absorbance values at 291 nm (λ_{max} of intact drug) and at 237 (λ_{max} of degradate) versus the concentrations in µg/ml were plotted to get the calibration graph and the regression equations was derived. Moxifloxacin hydrochloride concentrations were calculated using equation (3) mentioned under section "2.4".

4.1.5. Dual wavelength method:

The difference in the absorbance for the intact drug and its degradate was measured at 270 and 285 nm and plotted versus the concentrations in µg/ml to get the calibration graph and the regression equations was derived. Moxifloxacin hydrochloride concentrations were calculated from the corresponding regression equation.

4.2. Accuracy and Precision:

4.2.1. Intra-day precision:

Three concentrations of moxifloxacin hydrochloride were analyzed three times intraday using the previously mentioned procedures. The percentage of recoveries of each concentration of the drug and its relative standard deviation were calculated using the suggested methods.

4.2.2. Intermediate precision:

Three concentrations of moxifloxacin hydrochloride were analyzed on three successive days using the procedure stated under linearity. The percentage of recoveries of each concentration of the drug and its relative standard deviation were calculated using the suggested methods.

4.3. Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines **[50]** from the following equations:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

4.4. Application to laboratory prepared mixtures:

Laboratory prepared mixtures containing different ratios of moxifloxacin hydrochloride and its degradate within their calibration ranges were prepared. The spectra of these mixtures were recorded and the procedures under construction of calibration curves were then followed but using the recorded spectra of the prepared mixtures. Recoveries were calculated as previously mentioned in accuracy, and percentages of degradate in mixtures were calculated.

4.5. Application to pharmaceutical preparation:

Different concentrations within calibration range of each method were prepared from the solution of the pharmaceutical preparation, the spectra of these prepared concentrations were recorded and procedures under construction of calibration curves were followed using the recorded spectra of the pharmaceutical formulation prepared solution.
The validity of the methods was assessed by applying the standard addition technique.

5. RESULTS AND DISCUSSION

It was reported that accelerated degradation of moxifloxacin hydrochloride was achieved upon heating under reflux with 2M hydrochloric acid for 10 hours to give the decarboxylated product and the densitometric TLC was used for separation of the degradate [40]. The degradation product was illustrated in the **figure (2)**:

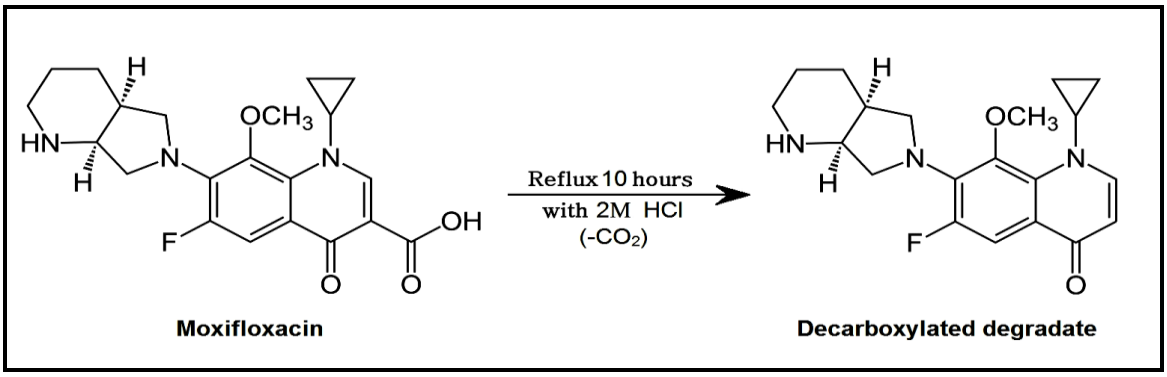

Fig. (2): Proposed degradation pathway of moxifloxacin.

By reviewing the literature, revealed the lack of any spectrophotometric methods for the determination of moxifloxacin hydrochloride in presence of its acidic-induced degradation product, the aim of this work is to develop five simple, accurate, precise methods for determination of moxifloxacin hydrochloride in presence of its degradation product without need of any pre-separation step or sophisticated instrument.

5.1. Bivariate method:

In order to apply the bivariate method the absorbance of the two components individually at seven different selected wavelengths was recorded in the region of overlapping; 260, 265, 270, 275, 280, 285 and 290 nm. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that; there was a linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient (r^2 > 0.9995). The yielded statistical results are summarized in table (1).

According to kaiser method, the slope values of the linear regression equations for both drugs at the selected wavelengths were used to calculate the sensitivity matrices (K) to find out the optimum pair of wavelengths at which the binary mixture was recorded. It was found that; the slopes at 275 and 290 nm gave the maximum value of K **table (2)** and thus chosen for the analysis.

Table (1): Linearity studies and regression equations of the proposed methods:

Parameters	Bivariate	Graphical absorbance ratio	Area under the curve	Simultaneous equation	Dual wavelength
Wavelength	275 & 290 nm	281.2 & 291 nm	(275-280) nm & (289-294) nm	237 & 291 nm	270 & 285 nm
Calibration range	(1-9 µg /ml)	(1-9 µg /ml)	(1-9 µg /ml)	(1-9 µg /ml)	(1-9 µg /ml)
Slope	0.1529	0.1548	0.7774	0.1548	0.0894
Intercept	0.0241	0.0172	0.0618	0.0172	-0.0109
Correlation coefficient	0.9998	0.9997	0.9999	0.9997	0.9997
LOD (µg /ml)	0.199	0.168	14 0.157	0.168	0.196
LOQ (µg /ml)	0.602	0.510	0.475	0.510	0.593

Table (2): The absolute values of the sensitivity matrix determinates calculated according to Kaiser's method (k x 10^5) for the mixture of moxifloxacin hydrochloride and its degradate:

λ/λ	260	265	270	275	280	285	290
260	0	64.07	208.67	105.18	417.45	712.35	1010.34
265		0	305.46	192.40	537.18	850.35	1141.02
270			0	125.20	240.65	626.69	1100.62
275				0	371.76	743.27	**1160.94**
280					0	432.16	1036.62
285						0	723.01
290							0

5.2. Graphical absorbance ratio method:

The absorption spectra of 6 µg/ml moxifloxacin hydrochloride, 6 µg/ml of its degradate, and a mixture containing equal concentration of them (3 µg/ml of each) showed isoabsorptive point at 281.2 nm,as shown in figure (3). The spectra show also isoabsorptive point at 309.4, 325.2 and 373.6 nm which were not involved in the method due to the low sensitivity of the drug at these wavelengths.

The absorbance values were measured at 291 nm (λ_{max} for moxifloxacin hydrochloride) and 281.2 nm (λ_{iso}) in the range of 1-9 µg/ml for both moxifloxacin hydrochloride and its degradate. Absorptivity coefficients were determined for both moxifloxacin hydrochloride and its degradate and the average values were taken. The values and the absorbance ratio were used to develop the previously mentioned sets of equations (8&9) under section "2.2" from which the concentration of moxifloxacin hydrochloride can be calculated. The yielded statistical results are summarized in table (1).

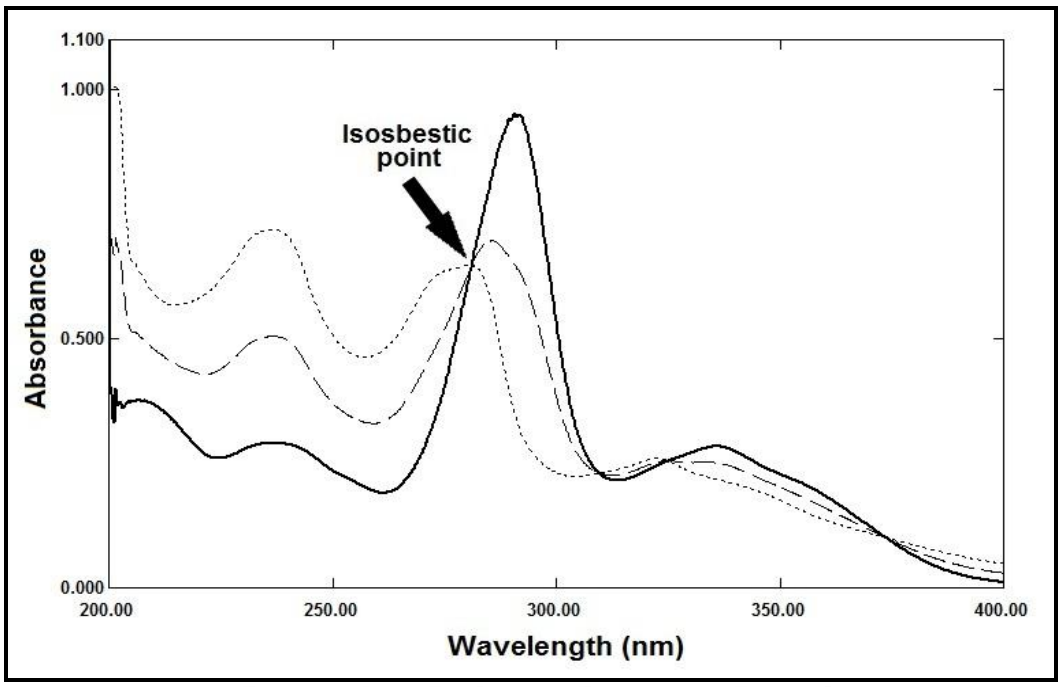

Fig. (3): Zero-Order Spectra of Intact moxifloxacin hydrochloride 6 µg/ml (—), its degradate 6 µg/ml (….) and their mixture 3 µg/ml of each (– –).

5.3. Area under the curve method:

In this method, the constant 'A' values of moxifloxacin hydrochloride and its degradate were calculated at each wavelength range, as shown in figure (4&5). The concentrations of moxifloxacin hydrochloride can be obtained by applying Cramer's rule and matrices in equations (1&2) under section "2.3". Concentration of intact drug in presence of its degradate was calculated according to the following equations:

$$AUC_{275-280} = 0.399\ C^{Moxifloxacin} + 0.532\ C^{Degradate} \qquad (1)$$

$$AUC_{289-294} = 0.780\ C^{Moxifloxacin} + 0.278\ C^{Degradate} \qquad (2)$$

Where, $C^{Moxifloxacin}$ and $C^{Degradate}$ are the concentrations of moxifloxacin hydrochloride and its degradate in µg/ml, respectively.

0.399 and 0.780 are constant 'A' of moxifloxacin hydrochloride at (275-280) and (289-294), respectively.

0.532 and 0.278 are constant 'A' of moxifloxacin hydrochloride degradate at (275-280) and (289-294), respectively.

$AUC_{275-280}$ and $AUC_{289-294}$ are the area under curve of sample solutions at wavelength range (275-280) and (289-294), respectively.

The yielded statistical results are summarized in table (1).

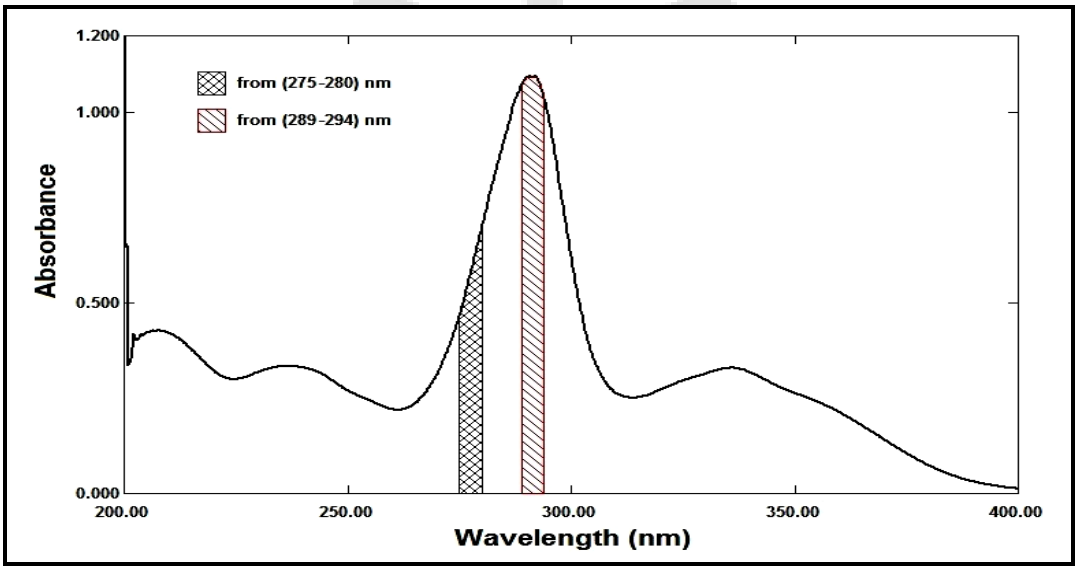

Fig. (4): Zero order spectrum of moxifloxacin hydrochloride 7 µg/ml showing area under the curve over the range (275-280) and (289-294) nm.

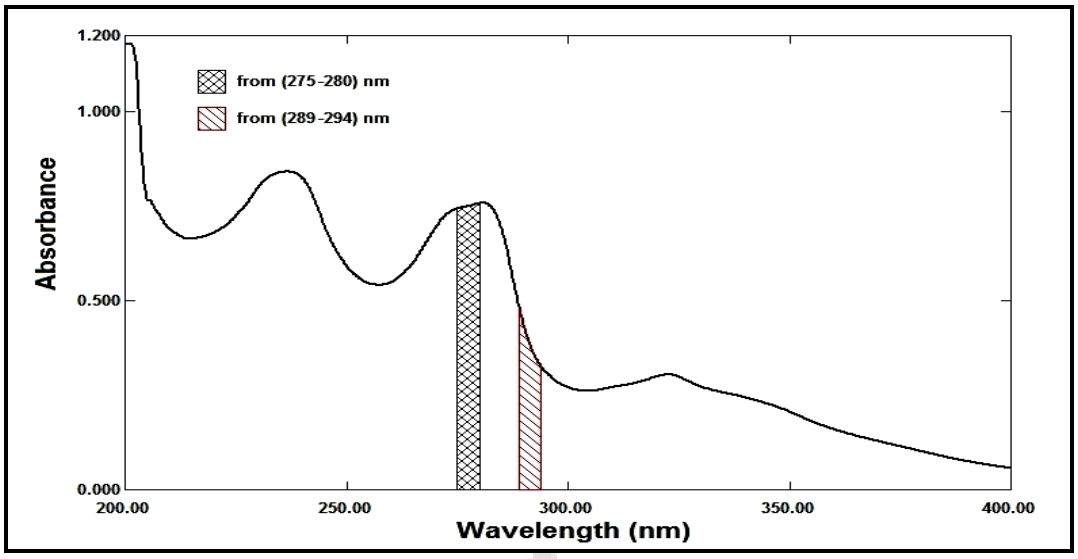

Fig. (5): Zero order spectrum of moxifloxacin hydrochloride degradate 7 µg/ml showing area under the curve over the range (275-280) and (289-294) nm.

5.4. Simultaneous equation method:

In this method, the absorptivity coefficients of the drug and its degradate at 291 nm and 237 nm, as shown in figure (6) were determined by dividing each absorbance over each corresponding concentration. The concentrations of moxifloxacin hydrochloride can be obtained by applying Cramer's rule and matrices in equations (1&2) under section "2.4". Concentration of moxifloxacin hydrochloride in presence of its degradate was calculated according to the following equations:

$$At\ \lambda_1 = 291 \quad A_1 = 0.157\ C_{Moxifloxacin} + 0.057\ C_{degradate} \qquad (1)$$

$$At\ \lambda_2 = 237 \quad A_1 = 0.048\ C_{Moxifloxacin} + 0.118\ C_{degradate} \qquad (2)$$

Where, $C_{Moxifloxacin}$ and $C_{degradate}$ are the concentrations of moxifloxacin hydrochloride and its degradate, respectively.

0.157 and 0.048 are the absorptivity of moxifloxacin hydrochloride at λ_1 and λ_2, respectively.

0.057 and 0.118 are the absorptivity of moxifloxacin degradate at λ_1 and λ_2, respectively.

A_1 and A_2 are the absorption values of sample solutions at λ_1 and λ_2, respectively.

The yielded statistical results are summarized in **table (1)**.

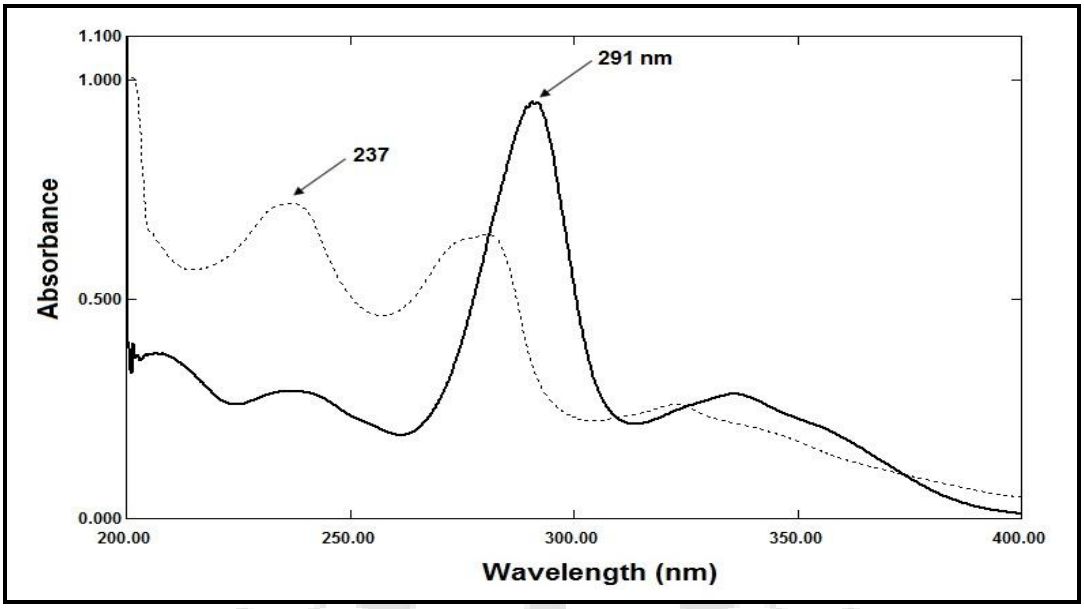

Fig. (6): Zero order spectra of moxifloxacin hydrochloride 6 µg/ml (—) with λ_{max} 291 nm and its degradate 6 µg/ml (....) with λ_{max} 237 nm.

5.5. Dual wavelength method:

In this method, the interference from the degradate can be removed by measuring the difference in absorbance at 270 and 285 nm. This difference is zero for the degradate, while it is directly proportional to the concentration of intact moxifloxacin hydrochloride, as shown in **figure (7)**. The yielded statistical results are summarized in **table (1)**.

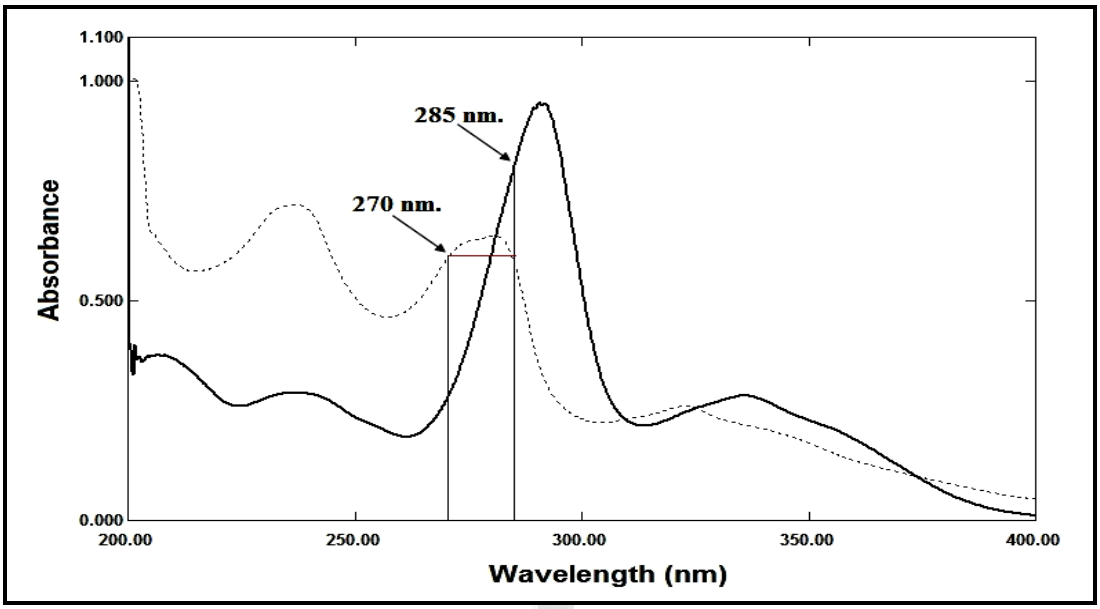

Figure (7): Zero order spectra of moxifloxacin hydrochloride 6 µg/ml (—) and its degradate 6 µg/ml (....) showing equal absorbance for moxifloxacin degradate at 270 nm and 285 nm.

5.6. Accuracy and Precision:

According to the ICH guideline, three replicate determination of three different concentration of the studied drug in pure form within their linearity ranges were performed on the same day (intra-day) and in three successive days (inter-day) for each method. Concentrations of (2, 5 and 7 ug/ml) were used in both bivariate and dual wavelength methods and concentrations of (4, 6 and 8 ug/ml) were used in graphical absorbance ratio, area under the curve and simultaneous equation method, and Accuracy as recovery percent (R%), and precision as percentage relative standard deviation (RSD%) were calculated and results were listed in table (3).

Table (3): Method validation obtained by applying the proposed methods:

Method	Conc. ($\mu g.ml^{-1}$)	Intraday		Interday	
		Accuracy (R%) ±SD	Precision (RSD%)	Accuracy (R%)±SD	Precision (RSD%)
Bivariate	2	99.65 ± 0.920	0.923	98.36 ± 0.343	0.349
	5	100.73 ± 0.585	0.581	100.15 ± 0.165	0.165
	7	99.42 ± 0.211	0.212	99.11 ± 0.683	0.689
Graphical absorbance ratio	4	101.79 ± 0.598	0.587	100.96 ± 1.104	1.093
	6	99.24 ± 0.637	0.642	98.75 ± 0.262	0.265
	8	99.07 ± 0.162	0.163	99.19 ± 0.830	0.836
Area under the curve	4	98.38 ± 0717	0.729	100.56 ± 0.298	0.296
	6	99.20 ± 0.433	0.436	101.45 ± 0.657	0.648
	8	101.16 ± 0.331	0.327	99.73 ± 0.601	0.603
Simultaneous equation	4	98.42 ± 0.379	0.385	99.56 ± 0.847	0.851
	6	100.37 ± 0.920	0.917	99.04 ± 0.955	0.964
	8	101.29 ± 0.280	0.276	98.11 ± 1.127	1.149
Dual wavelength	2	99.18 ± 0.729	0.735	101.09 ± 0.277	0.274
	5	99.52 ± 0.193	0.194	98.69 ± 0.909	0.921
	7	100.37 ± 0.816	0.813	99.48 ± 0.529	0.532

5.7. Specificity:

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of moxifloxacin hydrochloride and its degradate. The results were listed in table (4).

Table (4): Determination of intact moxifloxacin hydrochloride in laboratory prepared mixtures with its degradate by the proposed methods:

Conc. of moxifloxacin (µg/ml)	Conc. of degradate (µg/ml)	(%) of degradate	Recovery % of Intact				
			Bivariate	Graphical absorbance ratio	Area under the curve	Simultaneous equation	Dual wavelength
7	2	22.22	99.34	98.61	99.16	98.94	98.99
6	3	33.33	98.52	101.57	98.28	99.21	98.39
5	4	44.44	98.67	99.73	100.31	98.52	100.54
4	5	55.56	100.13	100.22	98.12	100.37	99.23
3	6	66.67	99.40	100.47	98.23	98.43	99.81
2	7	77.78	98.15	101.12	99.52	101.46	100.75
1	8	88.89	*96.07* [a]	*104.92* [a]	101.03	*93.87* [a]	101.29
Mean			99.04	100.27	100.43	99.49	99.24
RSD%			0.729	1.046	1.334	1.198	1.136

[a] Underlined values are out of accepted range and not considered in the calculation of Mean or SD.

5.8. Pharmaceutical Applications:

The proposed methods were applied to the determination of the studied drug in Moxiflox ® 400 mg tablets. The statistical comparison between the results obtained by applying the proposed methods and those obtained by applying the reported method [40] showed less calculated t and F values revealing no significant difference in accuracy and precision table (5). The validity of the methods was assessed also by applying the standard addition technique table (6).

Table (5): Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and reported methods for determination of moxifloxacin hydrochloride in Moxiflox® 400 mg tablets:

Parameter	Bivariate	Graphical absorbance ratio	Area under the curve	Simultaneous equation	Dual wavelength	Reported Method[40]***
Mean	100.03	100.62	100.54	99.54	100.42	99.38
S.D.	0.495	0.866	0.612	0.760	0.539	0.967
N*	5	5	5	5	5	5
t*-test*	1.326 (2.306)	2.133 (2.306)	2.256 (2.306)	0.287 (2.306)	2.093 (2.306)	———
F*-value*	3.809 (6.388)	1.246 (6.388)	2.498 (6.388)	1.619 (6.388)	3.218 (6.388)	———

* No. of experimental.

** The values in the parenthesis are the corresponding theoretical values of *t* and *F* at (*P* = 0.05).

*** Determination of moxifloxacin hydrochloride using first derivative [40].

Table (6): Application of standard addition technique to the analysis of Moxiflox® 400 mg tablets by applying the proposed methods:

Taken (µg /ml)	Added standard (µg /ml)	Recovery % of standard				
		Bivariate	Graphical absorbance ratio	Area under the curve	Simultaneous equation	Dual wavelength
3	2	99.15	100.68	101.79	99.20	100.71
	3	99.86	101.89	100.93	99.13	99.52
	4	100.22	99.75	98.34	100.72	98.14
	5	98.10	100.19	99.52	98.34	99.36
Mean		99.33	100.63	100.15	99.35	99.43
RSD%		0.941	0.918	1.522	1.001	1.057

6. Statistical comparative discussion of proposed methods:

All data mentioned above related to previous tables and figures introduce a comparative discussion for five techniques which applied for manipulating of moxifloxacin hydrochloride and its acidic degradation product. We know that "more small values of LOD and LOQ, more sensitive the methods", so according to LOD and LOQ area under the curve method seems to be more sensitive than the others, as shown in table(1). Graphical absorbance ratio method is the second one in sensitivity after area under the curve method, however, it requires isobestic point. On the other hand, simultaneous equation and dual wavelength methods seem to be more simple than other methods, since they do not need calculation of area under curve, calculation of Kaiser value or iso-absorptive point which involved in the other methods.

In table (4) we can note that area under the curve and dual wavelength methods were adopted for the selective determination of intact moxifloxacin hydrochloride in presence of up to 88.89% of its degradation product, while graphical absorbance ratio, simultaneous equation and bivariate methods were adopted for the selective determination of intact moxifloxacin hydrochloride in presence of up to 77.78% of its degradation product.

Statistical comparison of the results obtained by the proposed methods and official method was shown in table (5). The calculated [t and F] values were less than the theoretical ones indicating that there was no significant difference between the proposed and the official method with respect to accuracy and precision.

Finally, the proposed methods are simple without requirement for sophisticated technique or instruments, they also sensitive, selective and can be used for manipulation of moxifloxacin hydrochloride in its available dosage form.

7. CONCLUSION

This work introduced a comparative study using five valid, simple, accurate, sensitive, economic spectrophotometric methods for determination of moxifloxacin hydrochloride in presence of its acidic-induced degradation product without any separation steps or sophisticated instruments.

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