Analytical Chemistry Insights

ORIGINAL RESEARCH

Development and Validation of Spectrophotometric, Atomic Absorption and Kinetic Methods for Determination of Moxifloxacin Hydrochloride

Lobna M. Abdellaziz[1] and Mervat M. Hosny[2]

[1]Medicinal Chemistry Department, Faculty of pharmacy, Zagazig University, Egypt. [2]Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Egypt. Corresponding author email: mervat2200@yahoo.com

Abstract: Three simple spectrophotometric and atomic absorption spectrometric methods are developed and validated for the determination of moxifloxacin HCl in pure form and in pharmaceutical formulations. **Method (A)** is a kinetic method based on the oxidation of moxifloxacin HCl by Fe^{3+} ion in the presence of 1,10 o-phenanthroline (o-phen). **Method (B)** describes spectrophotometric procedures for determination of moxifloxacin HCl based on its ability to reduce Fe (III) to Fe (II), which was rapidly converted to the corresponding stable coloured complex after reacting with 2,2′ bipyridyl (bipy). The formation of the tris-complex formed in both methods (A) and (B) were carefully studied and their absorbance were measured at 510 and 520 nm respectively. **Method (C)** is based on the formation of ion- pair associated between the drug and bismuth (III) tetraiodide in acidic medium to form orange—red ion- pair associates. This associate can be quantitatively determined by three different procedures. The formed precipitate is either filtered off, dissolved in acetone and quantified spectrophotometrically at 462 nm (Procedure 1), or decomposed by hydrochloric acid, and the bismuth content is determined by direct atomic absorption spectrometric (Procedure 2). Also the residual unreacted metal complex in the filtrate is determined through its metal content using indirect atomic absorption spectrometric technique (Procedure 3). All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines, the three proposed methods permit the determination of moxifloxacin HCl in the range of (0.8–6, 0.8–4) for methods A and B, (16–96, 16–96 and 16–72) for procedures 1–3 in method C. The limits of detection and quantitation were calculated, the precision of the methods were satisfactory; the values of relative standard deviations did not exceed 2%. The proposed methods were successfully applied to determine the drug in its pharmaceutical formulations without interference from the common excipients. The results obtained by the proposed methods were comparable with those obtained by the reference method.

Keywords: moxifloxacin HCl, o-phenanthroline, bipyridyl, Bi(III)-iodide, kinetic, ion- pair, spectrophotometric

Analytical Chemistry Insights 2011:6 67–78

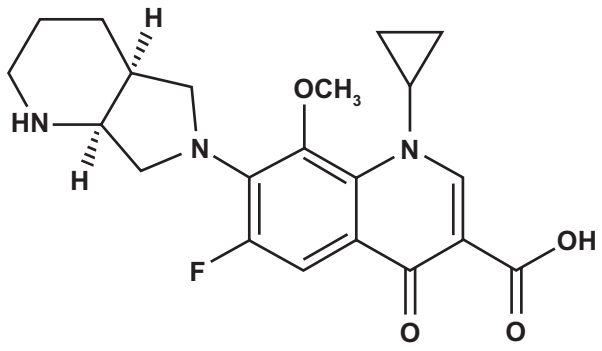
doi: 10.4137/ACI.S8090

This article is available from http://www.la-press.com.

Introduction

Moxifloxacin [1-Cyclopropyl-b-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo [3,4–6] pyridine-6-yl]-4-oxo-3-quinoline carboxylic acid][1] is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria.[2] The bactericidal activity of the drug is mediated by the inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, essential enzymes involved in bacterial DNA replication, transcription, repair and recombination. Moxifloxacin is prescribed for the bacterial infections of the respiratory tract including sinusitis, community acquired pneumonia and acute exacerbations of chronic bronchitis.[3]

HCl



Few methods were reviewed in the literature for the analysis of moxifloxacin HCl. Spectrophotometric,[4,5] spectrofluorimetric,[6] liquid chromatographic,[7–10] TLC,[11] HPLC,[12–16] Capillary electrophoresis,[17,18] polarographic,[19] and voltammetric[20,21] procedures were applied for its determination.

Still, there is a need for simple methods to compete with the new, advancement and automated ones. Therefore, the present study aims to use spectrophotometric and atomic absorption spectrometric (AAS) techniques for the determination of moxifloxacin HCl in pure form and pharmaceutical formulations. Methods A and B are based on the oxidation of the drug by Fe^{3+} in the presence of (o-phen) or (bipy) and then the tris-complex formed was measured at 510 and 520 nm respectively. While method C based on the precipitation of the ion pair of the drug with bismuth (III) tetraiodide and quantifying it via the formed precipitate of the metal ion present in the supernatant solution using (AAS). The proposed methods were successfully applied to the determination of moxifloxacin HCl in tablet dosage forms without interference of any additives or excipients.

Experimental Apparatus

A Shimadzu recording spectrometer UV-1800 equipped with 10 mm two matched quartz cells was used for spectrophotometric measurements. Atomic absorption measurements were carried out using Shimadzu atomic absorption spectrometric device model AA-640-13 at 223 nm analysis wavelength, lamp current 5 mA, slit width 0.38 nm, burner height 5 mm, burner slot, flame 10 cm air-C_2H_2, support gas flow 10 I min^{-1}, fuel gas flow 2.6 I min^{-1} and absorption sensitivity of 0.6 ppm.

Materials and Reagents

All materials used were of analytical reagent grade, water was always doubly distilled. Pure sample moxifloxacin HCl was kindly provided by Sabaa International Company for pharmaceuticals and chemical industries S.A.E.

The Standard stock solution (0.8 mg ml^{-1}) in water were prepared by dissolving 80 mg of pure drug in convenient amount of double distilled water in 100 ml volumetric flask followed by dilution to the mark with the same solvent, it is stable for at least 2 weeks if it was stored in a cool (<25 °C).

Pharmaceutical preparations: Moxifloxacin tablets (400 mg moxifloxacin HCl/tablet) were provided by Sabaa International Company for pharmaceuticals and chemical industries S.A.E. (Batch No. 09002).

Iron (III)-o-phenanthrolin[22] was prepared by mixing 0.198 g of 1,10 phenanthroline monohydrate (Aldrich Chem. Co. Miluwakee, USA), 2 ml 1 M HCl and 0.16 g ferric ammonium sulphate dodecahydrate (Aldrich, Germany) before dilution with double distilled water to 100 ml in a calibrated flask. Iron (III) – bipyridyl[22] was prepared by mixing 0.16 g of 2,2′ bipyridyl (Sigma Chem. Co. Miluwakee, USA) with 2 ml 1 M HCl and 0.16 g ferric ammonium sulphate dodecahydrate, before dilution with double distilled water to 100 ml in a calibrated flask.

Standard bismuth (III) solution, 0.01 M, was prepared by dissolving 0.1 g of $Bi(NO_3)$. $5H_2O$ (Merck) in 2.5 ml of HNO_3 and adding double distilled water to 25 ml and standardized complexometrically.[23]

Potassium iodide solution, 0.5 M, was prepared by dissolving 8.28 g of KI (Merck) in 100 ml of double distilled water. 2% HNO_3 solution was prepared in double distilled water. 0.8×10^{-3} M solutions of bismuth (III) nitrate and drug were prepared.

General Procedures

Methods A and B

(0.8–6) (0.8–4) μgml^{-1} aliquots of the standard solutions for methods A and B respectively were transferred to a series of 10 ml calibrated flasks. 4 ml of Fe^{3+}-o-phen (method A) or 3.5 ml of Fe^{3+}-bipy (method B) were added, then heating on a boiling water bath for 35, 30 minutes for methods A and B respectively. Mixture was cooled to room temperature (25 °C ± 1 °C), completed to volume with double distilled water. The coloured complexes formed were measured at 510 and 520 nm against a reagent blank treated similarly according to methods A and B respectively.

Procedures for the kinetic method

Aliquots of (0.8–6 μgml^{-1}) of moxifloxacin HCl were assayed as in the general procedure for method A at different times (10, 25, 40, 60 minutes) in a boiling water bath.

Method C

Procedure 1

To a series of 10 ml volumetric flasks, 0.7 ml of 0.01 M bismuth (III) nitrate solution was added followed by 0.8 ml of 0.5 M potassium iodide solution with continuous mixing. Accurately measured aliquots of the drug (Table 1) were then added followed by 0.7 ml of 2% HNO_3. The solution was shaken to coagulate the precipitate then completed to volume with double distilled water, mixed well, and filtered. The precipitate obtained was washed with 2 ml 2% HNO_3, completely dried in a vacuum desiccant, then dissolved quantitatively in acetone and the volume was made up to 10 ml in calibrated flask. The absorbance was measured at 462 nm against an appropriate blank prepared simultaneously.

Procedure 2

The precipitate in procedure 1 was quantitatively decomposed into 10 ml volumetric flasks using 1 ml concentrated HCl, the mixture was completed to 10 ml with double distilled water and aspirated directly in the atomic absorption spectrometer, absorption was measured at 2230 A° against an appropriate blank prepared simultaneously. Concentration of the consumed bismuth was calculated from a calibration graph of standard $Bi(NO_3)$ solution or using regression equation.

Procedure 3

The filtrate of procedure 1 were transferred to 25 ml volumetric flask, diluted to volume with double distilled water, absorbance was then measured using atomic absorption spectrometer at 2230 A° against an appropriate blank prepared simultaneously, excess

Table 1. Spectral data for determination of moxifloxacin HCl using methods (A, B and C).

Items	Method A	Method B	Method C		
			Procedure 1	Procedure 2	Procedure 3
Linearity range (μgml^{-1})	0.8–6	0.8–4	16–96	16–96	16–72
Apparent molar absorpitivity* ($L\ mol^{-1}\ cm^{-1}$)	6.61×10^4	8.5×10^4	4.5×10^3	1.2×10^6	1.2×10^6
Sandell's sensitivity** ($\mu g/cm^2$/0.001 Abs. unit)	0.0151	0.0196	1.03×10^{-3}	0.289	0.287
Intercept (a)***	0.10939	0.0266	0.08153	0.6215	0.6071
Slope (b)	0.11755	0.1862	0.0083174	2.879	2.8526
Correlation coefficient (r)	0.9998	0.9998	0.9999	0.9999	0.9998
Variance	0.74	0.50	0.57	0.69	0.73
Detection limit (μgml^{-1})	0.076	0.21	1.41	1.42	1.43
Quantification limit (μgml^{-1})	0.254	0.69	4.67	4.74	4.78

Notes: *Calculated on the basis of the molecular weight of the drug; **limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm; ***A = bX + a, Where A is the absorbance, X is concentration in μgml^{-1}, a is intercept, b is slope.

concentration was then determined from a calibration graph of standard $Bi(NO_3)$ solution or using regression equation. The concentration of the drug was then calculated where 15.27 μgml^{-1} Bi (III) ≡ 16 μgml^{-1} drug.

Procedure for dosage forms

An accurately weighed quantity of the pulverized tablets equivalent to 80 mg of the studied drug was extracted with double distilled water. Mixture was filtered through a filter paper and washed with water, the filtrate and washing were collected in a 100 ml standard flask and diluted to volume with double distilled water. Aliquots of this solution were transferred in a series of 10 ml volumetric flasks, and the analysis was completed as previously mentioned using methods A, B and C by using standard addition technique.

Results and Discussion

Methods A and B

1,10 (o-phen) and 2,2′(bipy) are common reagents for ferrous ion as their red color chelate $[Fe(phen)_3]^{2+}$, $[Fe(bip)_3]^{2+}$ complexes remained stable for weeks. These methods were based on the reducing properties of the drug, it reduces Fe(III) to Fe(II) which was converted rapidly to the corresponding stable coloured reagent Fe(II) complex. (Fig. 1).

Absorption spectra were measured at 510 and 520 nm for 1,10 (o-phen) and 2, 2′(bipy) respectively (Figs. 2 and 3).

Optimum conditions affecting the reaction were studied:

1. Effect of reagent volume: It was found that 4 and 3.5 ml of 1,10 (o-phen) and 2,2′(bipy) respectively were suitable to give optimum results with moxifloxacin HCl.
2. Effect of temperature and heating time: At ambient temperature (25 °C ± 2 °C), the reaction was very slow, when temperature increases, the reaction was faster until it reach the maximum absorbance in a boiling water bath (100 °C). Heating for 35–30 minute in a boiling water bath gave the maximum absorbance for methods A and B respectively.
3. Effect of solvent: Different solvents such as water, ethanol and isopropyl alcohol were tried in dilution, water was found to be the most suitable solvent.

After diluting the reaction solution mixture, it was found that the absorbance of the chromogen formed in method A and B are remained stable for at least 2 hours. This allowed the processing of large batches of samples, and their comfortable measurements with convenience so the methods will be more applicable for large number of samples, calibration graphs obtained were found to be linear over concentration ranges stated in (Table 1).The linearity was evaluated by the relative standard deviation of the slope,[24] standard error, variance, Sandell's sensitivity were also calculated (Tables 1 and 2).

Results and discussion of kinetic spectrophotometric procedure for determination of moxifloxacin with 1,10 (o-phen) and $FeCl_3$

The rate of the reaction was found to be dependent on the drug concentration, the rate was followed at 100 °C with various concentrations of the studied drug in the range of (0.8–6) μgml^{-1} (Fig. 4).

It is clear that the rate increases as the studied drug concentration increases, indicating that the reaction rate obeys the following equation:

$$\text{Rate} = K' [\text{drug}]^n \quad (1)$$

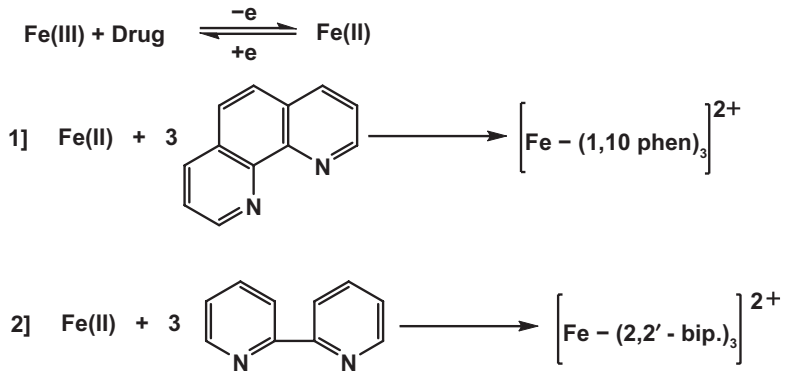


Figure 1. Reactions of moxifloxacin HCl with 1,10 (o-phen) and 2, 2′(bipy).

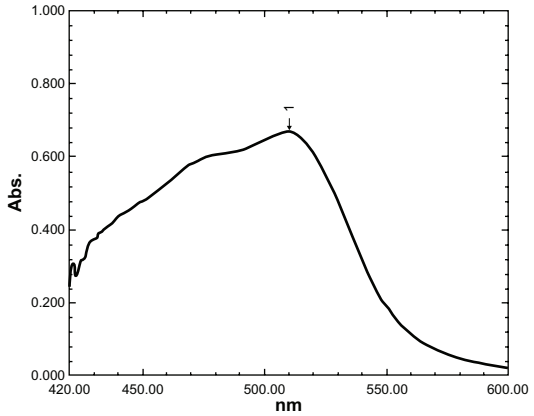


Figure 2. Absorption spectra of the complex formed through reaction of 4.8 μgml^{-1} Moxifloxacin HCl with 1,10 phenanthrolin and $FeCl_3$.

where K' is the pseudo-order constant of the reaction and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method measurement[25] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentration (Table 3) equation (1) is transformed into:

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}] \quad (2)$$

Regression of log (rate) versus log (drug) gave the regression equation:

$$\log (\text{rate}) = -0.750 + 0.541 \log C \quad (r = 0.969),$$

$$K' = 5.623\ \text{S}^{-1}$$

Hence the reaction is first order ($n \approx 1$) with respect to drug concentration.

Evaluation of the kinetic methods

The quantitative of the studied drug under the optimized experimental conditions outlined above, would result in a pseudo-first order reaction with respect to its concentration. However, the rate will be directly proportional to drug concentration in a pseudo-first order rate equation as follows:

$$\text{Rate} = K' [\text{drug}] \quad (3)$$

where K' is the pseudo-first order constant. Equation (3) was the basis for several experiments, which were run to obtain drug concentration using the rate data. Rate constant, constant concentration and fixed-time.[26,27] The most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept.

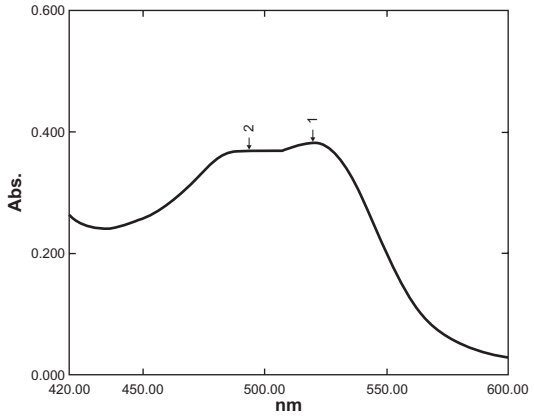


Figure 3. Absorption spectra of the complex formed through reaction of 1.8 μgml^{-1} Moxifloxacin HCl with 2,2′ bipyridyl and $FeCl_3$.

Rate-constant method

Graphs of log (absorbance) versus time for the studied drug concentrations in the range (1.826×10^{-6} to 1.370×10^{-5} M) was plotted and appeared to be rectilinear. Pseudo-first order rate constants corresponding to different drug concentrations (C) were calculated from the slopes multiplied by –2.303 and are presented in (Table 4), (Fig. 5).

Regression of (C) versus K' gave the equation:

$$K' = 1.2999 \times 10^{-5} + 0.015532\ C \quad (r = 0.9619) \quad (4)$$

The value (r) indicates poor linearity, which is probably due to inconsistency of K' as a result of slight changes due to the elevated temperature of the reaction.

Fixed-concentration method

Reaction rates were determined for different concentrations in the range 1.826×10^{-6} to 1.370×10^{-5} M of moxifloxacin HCl. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time (ie, $1/t$) versus the initial concentration of the studied drug (Table 5) was plotted. The following equations for calibration graphs were worked out by linear regression:

$$1/t = 5.204 \times 10^{-7} + 8.0241 \times 10^{-3}\ C \quad (r = 0.99838) \quad (5)$$

The range of the concentration of the studied drug giving the most acceptable calibration graph with the above equations was very limited, which could be disadvantage.

Table 2. Determination of moxifloxacin HCl using methods A and B.

Method A		Method B	
Taken (μgml^{-1})	**Recovery %**	**Taken (μgml^{-1})**	**Recovery %**
0.8	99.54	0.8	99.62
2.4	99.46	2	100.62
3.2	101.18	2.8	100.58
5.2	99.07	3.6	98.96
6	100.47	4	100.61
Mean* ± Standard deviation ($P = 0.05$)	99.94 ± 0.8610	100.01 ± 0.7074	
N	5	5	
Variance	0.7413	0.5004	
Standard error	0.3850	0.316	

Note: *Mean of three different experiments.

Fixed time method

Reaction rates were determined for different concentration of the studied drug, at a pre-selected fixed time, which was accurately determined, the absorbance was measured. Calibration graph of the absorbance versus initial concentration of the moxifloxacin HCl was obtained at fixed times of 10, 25, 35 and 40 min with the calibration equation shown in (Table 6). It is clear that, the most acceptable values of the correlation coefficient and more reaction products (indicated by higher absorbance readings were obtained for a fixed time of 35 min, which was, therefore chosen as the most suitable time interval for measurements.

After optimizing the reaction conditions, the fixed time method was applied to the determination of moxifloxacin hydrochloride in pure form and in pharmaceutical formulations over the concentration range of (0.8–6 μgml^{-1}). Analysis of the date gives the following regression equation:

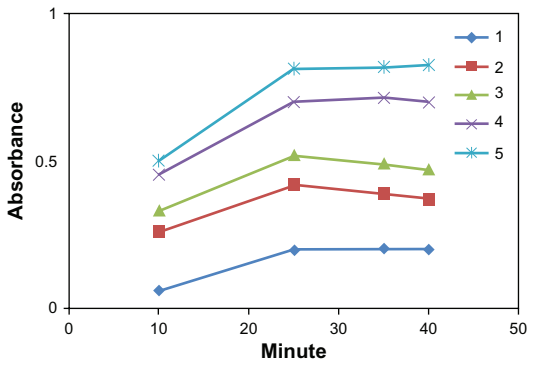


Figure 4. Absorbance versus time graphs for the reaction between moxifloxacin HCl and 1,10 phenanthrolin showing the dependance of the reaction on drug concentration (1) 1.826×10^{-6}; (2) 5.48×10^{-6}; (3) 7.307×10^{-6}; (4) 1.187×10^{-5}; (5) 1.370×10^{-5}.

$$A = 0.10939 + 0.11755\ C \quad (r = 0.9998) \qquad (6)$$

Method C

Formation of ion- pairs between many nitrogenous drugs and metal complexes found wide applications in the field of drug analysis applying colourimetric and (AAS) methods as well as ion-selective electrodes.[28–37] In this work the investigated drug react with bismuth (III) tetraiodide to form stable ion- pair complex, this interaction and subsequent formation of the ion- pair occur in acidic medium via the two centers of tertiary amino group of the drug, one of them was formed due to the addition of acid) and two molecules of bismuth (III) tetraiodide. Being insoluble in aqueous solution, this ion- pair complex might be possibly isolated by direct filtration or extraction into organic solvents and assayed accurately without interference from excess unreacted metal complex.

Bismuth (III) tetraiodide complex was used as a reagent for the determination of some nitrogenous compounds.[35–37] On mixing aqueous solutions of bismuth (III) tetraiodide complex and drug in acidic medium, a reddish orange precipitate instantaneously

Table 3. Logarithms of the rates for different concentrations of moxifloxacin hydrochloride at constant concentration of 1,10 (o-phen) and $FeCl_3$.

Log (rate), log $\Delta A/\Delta t$	Log (drug) (M)
–3.82	–5.74
–3.64	–5.26
–3.59	–5.14
–3.38	–4.93
–3.35	–4.86

Table 4. Values of *K′* calculated from slope of log *A* vs. *t* graphs multiplied by –2.303 for different concentrations of moxifloxacin hydrochloride and constant concentration of reagent.

K′ (S^{-1})	(M)
-7.5943×10^{-4}	1.826×10^{-6}
-4.1338×10^{-4}	5.480×10^{-6}
-3.396×10^{-4}	7.307×10^{-6}
-1.5966×10^{-5}	1.187×10^{-5}
-9.562×10^{-5}	1.370×10^{-5}

appeared that is attributed to the ion- pair formed in the reaction. This precipitate was filtered off and the residual unreacted bismuth (III) tetraiodide complex in the filtrate was analyzed using atomic absorption spectrometric technique. On the other hand the precipitate could be dissolved in acetone and analyzed spectrophotometrically at its peak absorption maximum at 462 nm (Fig. 6) or dissolved in HCl for atomic absorption spectrometric estimation.

Extraction of the formed ion- pair with different solvents was also studied, low polarity solvents such as chloroform and dichloromethane were inefficient due to insolubility of the ion- pair in such solvents, solvents with increase polarity such as n-butanol, acetone, and isobutyl methyl ketone lacked selectivity and did not differentiate between the ion- pair formed and the residual unreacted bismuth (III) tetraiodide in the aqueous phase, therefore filtration was necessary to separate the formed ion- pair.

Determination of the residual non-consumed bismuth (III) tetraiodide complex in the filtrate had the advantages of being rapid, simpler, precise than the direct dissolution of the isolated ion- pair precipitate

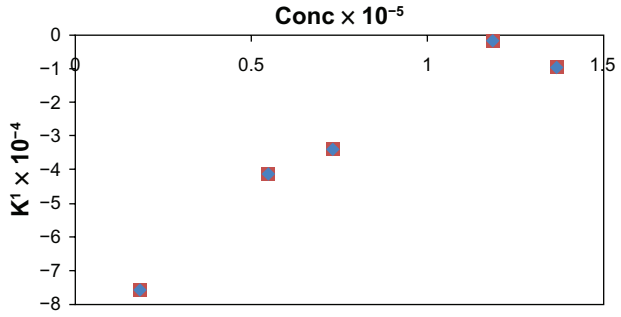


Figure 5. Values of *K′* calculated from slopes of different log *A* vs. *t* graph multiplied by –2.303 for different concentrations of drug at constant concentration of reagent.

Table 5. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentration of moxifloxacin hydrochloride.

1/*t* (S^{-1})	(M)
1.66×10^{-3}	1.370×10^{-5}
1.388×10^{-3}	1.187×10^{-5}
8.547×10^{-4}	7.307×10^{-6}

owing to possible errors during isolation steps, automation of the methods enhance the overall analytical progress of the proposed procedure, making them more suitable for routine quality control analysis of the studied drug.

The different experimental parameters affecting the formation of the ion- pair complex were studied to determine the optimum conditions for the assay procedure:

1. Effect of reagents volume: It was found that 0.7 ml of 0.01 M bismuth nitrate and 0.8 ml of 0.5 M potassium iodide were required to obtain the maximum precipitation of the drug as its ion- pair.
2. Effect of acid concentration: The choice of a suitable pH value at which the ion associate exhibit the lowest solubility (at 25 °C) is of prime importance in the use of such compounds in quantitative analysis. To determine this pH value, 0.05 M of KOH or 2% HNO_3 was used to adjust the pH of the solution, from the obtained results, it was observed that at pH $>$ 3, there is a decrease in the precipitation yield with increase in pH, probably because of precipitation of bismuth as a hydroxo-species. The absorbance was maximum at pH 1.7 which was achieved by using 0.7 ml 2% HNO_3.

Generally the formation of the ion- pair was rapid and the colour production was still stable for

Table 6. Calibration equations for moxifloxacin hydrochloride at different fixed time over the range of 1.826×10^{-6} –1.370×10^{-5} M in presence of constant concentration of reagent.

Time (min)	Calibration equation	Correlation coefficient (*r*)
10	$A = 0.03486 + 0.0813\ C$	0.9818
25	$A = 0.1295 + 0.1136\ C$	0.9959
35	$A = 0.10939 + 0.11755\ C$	0.9998
40	$A = 0.0934 + 0.1192\ C$	0.9986

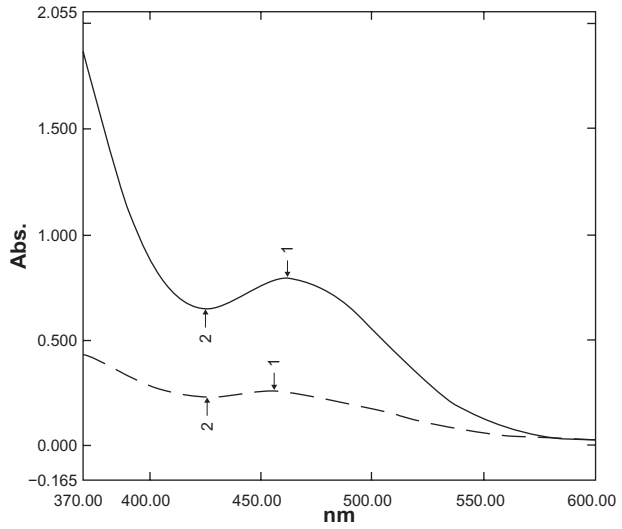


Figure 6. Absorption spectra of Bi(III)- 88 μgml^{-1} moxifloxacin ion pair (—) versus reagent blank (---).

more than 24 hour. The more favorable sequence addition is bismuth (III)-KI- drug for the highest and completion- ion- pair formation. The linearity was evaluated by the relative standard deviation of the slope, standard error, variance, Sandell's sensitivity. (Tables 1 and 7).

Composition of the ion- pair associates

The composition of the ion- pair associates was established by molar ratio method.[38] using equimolar solutions of the drug and reagent (0.8×10^{-3}), the results obtained indicate that the composition of the associates was (1:2) drug to reagent. According to this ratio it was found that 15.27 μgml^{-1} Bi (III) ≡ 16 μgml^{-1} moxifloxacin HCl.

Methods of Validation

Under the experimental conditions described above the optical characteristics such as Beer's law limits, Sandell's sensitivity and molar absorptivity[39] were calculated for the proposed methods and the results are summarized in (Table 1). Regression equations, intercepts, slopes and correlation coefficients for the calibration data are presented also in the same table while standard deviation, relative standard deviation and standard error are summarized in (Tables 2 and 7).

Sensitivity

The detection limit (*LOD*) for the proposed methods were calculated using the following equation according to definition[40]:

$$LOD = 3s/k \quad (7)$$

where s is the standard deviation of replicate determination values under the same conditions as the sample in the absence of the analyte, and k is the sensitivity, namely the slope of the calibration graph. The detection limits obtained for the absorbance were found to be 0.0762, 0.21, 1.41, 1.423, 1.436 μg/ml for methods A, B, and C (Procedures 1, 2 and 3), respectively.

The limits of quantitation, *LOQ*, is defined as;

$$LOQ = 10s/k \quad (8)$$

Table 7. Determination of moxifloxacin HCl using method C (Procedures 1, 2 and 3).

	Recovery %		
	Procedure 1	**Procedure 2**	**Procedure 3**
	98.79	98.51	99.46
	100.93	100.27	98.44
	99.81	99.32	100.12
	100.79	100.85	100.25
	99.09	100.71	101.07
	99.94	100.41	100.41
	100.46	99.99	100.45
	99.87		99.03
Mean* ± standard deviation ($P = 0.05$)	99.96 ± 0.7593	100.01 ± 0.8316	99.90 ± 0.860
N	8	7	8
Relative standard deviation	0.7596	0.8315	0.861
Variance	0.5765	0.6915	0.7396
Standard error	0.268	0.314	0.304

Note: *Mean of three different experiments.

Table 8. The inter-day precision and accuracy data for moxifloxacin HCl obtained by the proposed methods.

Method	Added µgml^{-1}	Intra-day			Inter-day		
		Found ± SE* µgml^{-1}	Precision RSD %	Accuracy R.M.E. %	Found ± SE* µgml^{-1}	Precision RSD %	Accuracy R.M.E. %
A	1.2	1.18 ± 0.57	1.42	–1.05	1.20 ± 0.39	0.96	0.47
	1.6	1.62 ± 0.11	0.26	1.5	1.61 ± 0.29	0.72	0.62
	2.6	2.62 ± 0.17	0.43	0.96	2.62 ± 0.19	0.46	0.76
B	1.6	1.61 ± 0.17	0.42	0.79	1.60 ± 0.20	0.51	0.18
	2.4	2.42 ± 0.13	0.31	1.08	2.42 ± 0.37	0.91	0.83
	3.2	3.21 ± 0.26	0.64	0.59	3.22 ± 0.29	0.71	0.67
C							
Procedure 1	32	31.98 ± 0.46	1.15	–0.06	32.04 ± 0.49	1.20	0.12
	64	64.44 ± 0.309	0.7528	0.682	64.46 ± 0.31	0.750	0.72
	72	71.97 ± 0.342	0.839	–0.041	71.90 ± 0.360	0.884	–0.1388
Procedure 2	24	23.64 ± 0.722	1.7967	–1.5	23.58 ± 0.620	1.546	–1.763
	40	40.08 ± 0.501	1.2246	0.2	39.96 ± 0.579	1.421	–0.1
	56	56.45 ± 0.296	0.719	0.803	56.4 ± 0.357	0.868	0.714
Procedure 3	48	47.98 ± 0.452	1.107	–0.041	47.92 ± 0.485	1.189	–0.166
	56	56.52 ± 0.468	1.137	0.928	56.34 ± 0.350	0.854	0.607
	64	53.59 ± 0.315	0.777	–0.640	63.59 ± 0.315	0.777	–0.641

Note: *Average of 6 determinations.

According to this equation, the LOQs were found to be 0.254, 0.6981, 4.676, 4.744, 4.788 µg/ml for methods A, B, and C (Procedures 1, 2, and 3), respectively.

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing 3 different concentrations of drug were prepared and analyzed in six replicate. The relative standard deviation as precision percentage relative error (Er %) as accuracy of the suggested methods were calculated at 95% confidence levels and can be considered satisfactory. Precision was carried out by six determinations at three different concentrations, the percentage relative error was calculated according to the following equation:

$$\text{Er \%} = [(\text{found} - \text{added})/\text{added}] \times 100$$

The inter- and intra-day precision and accuracy results are shown in (Table 8). The analytical results for accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables including, concentration of analytical reagents, reaction time. In these experiments, one parameter was changed where as the others were kept unchanged, It was found that none of these variables significantly affect the method. This provided as indication for the reliability of the proposed method during its routine application for analysis of the investigated drug. Ruggedness was tested by applying the proposed methods to the assay of the drug using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained were found to be reproducible, as RSD did not exceed 2% (Table 9).

The % recoveries of the pure drug using the proposed methods were compared with that given by the reference method[4] are illustrated in (Table 10). The reference method recomonded is uv-spectrophotometric procedure for determination of moxifloxacin hydrochloride using

Table 9. Results of evaluation of the ruggedness of the proposed spectrophotometric methods for determination of moxifloxacin HCl.

Method	Shimadzu UV-1800	Shimadzu UV-260
Method A	99.94 ± 0.861	98.7466 ± 1.0103
Method B	101.01 ± 0.707	101.65 ± 1.225
Method C Procedure 1	99.96 ± 0.7593	100.99 ± 1.2438

Note: *Recovery % ± SD.

Table 10. Statistical data for the determination of moxifloxacin HCl using method (A–C) compared with reference method.

Item	Reference method	Method A	Method B	Method C		
				Procedure 1	Procedure 2	Procedure 3
Mean ± S.D. ($P = 0.05$)	101.03 ± 1.2	99.94 ± 0.8610	100.01 ± 0.7074	99.96 ± 0.7593	100.01 ± 0.8316	99.90 ± 0.860
N	9	5	5	8	7	8
R.S.D	1.276	0.8615	0.70737	0.7596	0.8315	0.861
V	1.664	0.7413	0.50041	0.5765	0.6915	0.7396
t	–	1.67 (2.17)	0.5036 (2.17)	2.047 (2.131)	0.460 (2.145)	2.094 (2.131)
F	–	2.24 (3.84)	3.325 (3.69)	2.886 (3.60)	2.406 (3.58)	2.249 (3.60)

Note: *Values in parentheses indicate theoretical values of t and F at $P = 0.05$.

Table 11. Application of standard addition technique for the determination of moxifloxacin HCl in pharmaceutical preparations using methods A and B.

Method A			Method B		
Taken (μgml^{-1})	Added (μgml^{-1})	Recovery %	Taken (μgml^{-1})	Added (μgml^{-1})	Recovery %
0.8	–	97.42	0.8	–	101.63
	0.8	98.48		1.2	99.08
	1.6	98.16		1.6	98.81
	2.4	99.11		2	100.80
	3.2	100.65		2.8	100.58
	5.2	98.75		3.2	100.42
Mean* ± SD	99.03 ± 0.9706		99.94 ± 0.9214		
N	5		5		
Relative standard deviation	0.9801		0.9215		
Variance	0.9421		0.848		

Note: *Mean of three different experiments.

Table 12. Application of standard addition technique for the determination of moxifloxacin HCl in pharmaceutical preparations using method C (Procedures 1, 2 and 3).

Procedure 1			Procedure 2			Procedure 3		
Taken (μgml^{-1})	Added (μgml^{-1})	Recovery %	Taken (μgml^{-1})	Added (μgml^{-1})	Recovery %	Taken (μgml^{-1})	Added (μgml^{-1})	Recovery %
24	–	98.03	24	–	98.96	24	–	96.30
	16	98.03		32	96.26		16	99.46
	24	97.42		40	96.87		32	99.02
	32	98.62		56	96.48		40	101.13
	48	98.56		64	98.27		48	100.34
	56	98.87						
	72	99.11						
Mean* ± SD	98.44 ± 0.61		96.97 ± 0.90			99.99 ± 0.9387		
N	6		4			4		
SD	0.6146		0.902			0.9387		
RSD	0.6243		0.930			0.9387		
V	0.3777		0.813			0.88115		

Note: *Mean of three different experiments.

0.1 N HCl. The validity of the proposed methods was evaluated by statistical analysis[41] between the results obtained and that of reference method. Regarding the calculated student's, *t*-test and variance ratio F-test, there is no significant difference between the proposed methods and the reference one.

Application

The proposed methods were successfully applied to determine moxifloxacin HCl in its commercial tablets using standard addition technique (Tables 11 and 12).

Conclusion

The proposed methods described in this paper are simple, economic, sensitive, don't require expensive reagents and sophisticated instruments. These methods are applicable for routine analysis of the studied drug in raw materials and pharmaceutical formulations over wide concentration range without interference from common excipients. The methods can use both spectrophotometric and (AAS) techniques for the final measurement step, moreover, they also have the advantages that no extraction is needed to separate the ion- pair formed and so avoiding the hazards of the organic solvents being simpler and more convenient. The statistical parameters indicate the reproducibility and accuracy of the methods.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

References

1. The Merck index. An encyclopedia of Chemicals, *Drugs and biologicals*. 2006;14th ed.,6291.
2. Balfour JAB, Lamb HM. *Drugs*. 2000;59:115–39.
3. Muijsers RBR, Jarvis B. *Drugs*. 2002;62:967–73.
4. Motwani KS, Shruti C, Farhan JA, Roop KK. Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations. *Spectrochim Acta Part A*. 2007;68:250–6.
5. Kaur K, Kumar A, Malik AK, Singh B, Rao AL. Spectrophotometric methods for the determination of fluoroquinolones: *A Review. Critical Reviews in Analytical Chemistry*. 2008;38(1):2–18.
6. Ocana JA, Barragan FJ, Callejon M. Spectrofluorimetric determination of moxifloxacin in tablets, human urine and serum. *The Analyst*. 2000;125(12): 2322–5.
7. Zhang H, Ren YP, Bao XL. Simultaneous determination of (fluoro) quinolones antibacterials residues in bovine milk using ultra performance liquid chromatography-tandem mass spectrometry. *J of Pharm and Biomed Anal*. 2009;49(2):367–74.
8. De Smet J, Boussery K, Colparet K, et al. Pharmacokinetics of fluoroquinolones in critical care patients: A bio-analytical HPLC method for the simultaneous quantification of ofloxacin, ciprofloxacin and moxifloxacin in human plasma. *J Chromatogr B Analytical Technologies in the Biomedical and Life Sciences*. 2009;877(10):961–7.
9. Hemanth Kumar K, Ramach G. Simple and rapid liquid chromatography method for determination of moxifloxacin in plasma. *J of Chromatogr B: Analytical Technologies in the Biomedical and life Sciences*. 2009;877(11–2): 1205–8.
10. Patil KR, Rane VP, Sang Shelti JN, Shinde DB. Stability-indicating LC method for analysis of lornoxicam in the dosage form. *Chromatographia*. 2009;69(9/10):1001–5.
11. Motwani SK, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar S. Application of a validated stability—indicating densitometric thin—layer chromatographic method to stress degradation studies on moxifloxacin. *Anal Chim Acta*. 2007;582(1):75–82.
12. Ulu ST. High-performance liquid chromatography assay for moxifloxacin: pharmacokinetics in human plasma. *J Pharm and Biomed Anal*. 2007;43(1): 320–4.
13. Srinivas N, Narasu L, Shankar BP, Mullangi R. Development and validation of a HPLC method for simultaneous quantitation of gatifloxacin, sparfloxacin and moxifloxacin using levofloxacin as internal standard in human plasma: application to a clinical pharmacokinetic study. *Biomedical Chromatography*. 2008;22(11):1288–95.
14. Baietto L, Apos D, Avolio A, et al. Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. *Therapeutic Drug Monitoring*.2009;31(1):104–9.
15. Djurdjevic P, Ciric A, Djurdjevic A, Stankov MJ. Optimization of separation and determination of moxifloxacin and its related substances by RPHPLC. *J Pharm and Biomed Anal*. 2009;50(2):117–26.
16. Sultan MA. New, simple and validated kinetics spectrophotometric method for determination of moxifloxacin in its pharmaceutical formulations. *Arabian Journal of Chemistry*. 2009;2:79–85.
17. Yang YG, Qin WD. Separation of fluoroquinolones in acidic buffer by capillary electrophoresis with contactless conductivity detection. *J Chromatogr A*. 2009;1216(27):5327–32.
18. Fan YX, Tian Z, Qin WD. Quick and sensitive determination of fluoroquinolones by capillary electrophoresis-potential gradient detection. *Anal Lett*. 2009;42(7–9):1057–69.
19. Abdel Ghani NT, El-Ries MA, El-Shall MA. Validated polarographic methods for the determination of certain antibacterial drugs. *Anal Sci*. 2007;23(9): 1053–8.
20. Erk N. Voltammetric behaviour and determination of moxifloxacin in pharmaceutical products and human plasma. *Anal and Bioanal Chem*. 2004;378(5):1351–6.
21. Trindade MAG, da Silva GM, Ferreira VS. Determination of moxifloxacin in tablets and human urine by square-wave adsorptive voltammetry. *Microchemical Journal*. 2005;81(2):209–16.
22. Amin AS, Zaky M, Khater HM, El-Beshbeshy AM. New colorimetric methods for microdetermination of melatonin in pure and in dosage forms. *Anal Lett*. 1999;32:1421–33.
23. Vogel AIA. *Text Book of Quantitative Inorganic Analysis*. 3rd ed. Longman: London; 1961:442.

24. Torrado S, Torrrado S, Cadoringa R. Comparison of assay methods by second-derivative spectroscopy, colorimetry and fluorescence spectroscopy of salicylic acid in aspirin preparations with a high-performance liquid chromatographic method. *J Pharm Biomed Anal*. 1994;12(3):383–7.
25. Weisberger A, Friess SL, Lewis ES. Techniques of Organic Chemistry, vol. 3, Interscience, New York, Part III. 1953.
26. Yatsimirskii KB. Kinetic Methods of Analysis, Pegamon Press, Oxford; 1966.
27. Laitinen HA, Harris WE. *Chemical Analysis*. 2nd ed. McGraw-Hill, New York; 1975.
28. El-Ansary AL, El-Hawary WF, Issa YM, Ahmed AF. Application of ionpairs in pharmaceutical analysis. atomic absorption spectrometric determination of promazine, chlorpromazine, imipramine and ciprofloxacin hydrochlorides with sodium cobaltinitrite. *Anal Lett*. 1999;32:2255–69.
29. Khalil S, Kelzieh A. Determination of verapamil in pharmaceutical formulations using atomic emission spectrometry. *J Pharm Biomed Anal*. 2002;27:123–31.
30. Fujiwara T, Mohammadzai IU, Murayama K, Kumamaru T. Solvent extraction coupled on-line to a reversed micellar mediated chemiluminescence detection system for trace-level determination of atropine. *Anal Chem*. 2000;72,1715–9.
31. Shoukry AF, Issa YM, Ibrahim H, Mohamed SK. Atomic emission spectrometric determination of antazoline, hydralazine and amiloride hydrochlorides, and quinine sulfate based on formation of ion associates with manganese thiocyanate. *Analyst (Cambridge, UK)*. 1995;120:1211–3.
32. Magnuszewska B, Figaszewski Z. Application of promazine compound with cadmium tetraiodide complex in the liquid membrane of an ion-selective electrode. *Chem Anal (Warsaw)*. 1998;43:265–73.
33. El-Kousy NM, Bebawy LI. Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods. *J Pharm Biomed Anal*. 1999;20:671–9.
34. Abdel-Gawad FM, El-Guindi NM. Spectrophotometric determination of metoclopramide and oxybuprocaine through ion pair formation with thiocyanate and Molybdenum(V) or Cobalt(II). *Anal Lett*. 1995;28:1437–47.
35. Abdel-Gawad, FM. Ion- pair formation of Bi(III)–iodide with some nitrogenous drugs and its application to pharmaceutical preparations. *J Pharm Biomed Anal*. 1998;16,793–9.
36. Eisman M, Gallego M, Valcarcel M. Indirect flame atomic absorption spectrometric determination of papaverine, strychnine and cocaine by continuous precipitation with Dragendorff's reagent. J *Anal At Spectrom*. 1993;8:1117–20.
37. Kar A. Spectrophotometric determination of mebendazole in pure and dosage forms by complexation with potassium bismuth(III) iodide. *Analyst (London)*. 1985;110:1031–3.
38. Job P. Spectrochemical Methods of Analysis. Wiley Intersience: New York; 1971:346.
39. Zavis H, Ludvik D, Milan K, Ladislaw S, Frantisck V. Handbook of Organic Reagents in Inorganic Analysis. Translated by Stanislav, K, Dr. Chalmers (The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester), A Division of John Wiley & Sons IC, New York, London, Sydney, Toronto; 1976:P364.
40. Lurie J. Hand Book of Analytical Chemistry. Mir publishers: Moscow; 1975.
41. Caulcut R, Boddy R. Statistics for Analytical Chemists. Chapmann & Hall: London; 1983.