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Development and Validation of Spectrophotometric Method for Determination of Moxifloxacin HCL in Bulk and Pharmaceutical Formulations

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ABSTRACT:

A Simple, rapid, and sensitive spectrophotometric method was developed for the determination of a fluoroquinolone antibiotic: moxifloxacin hydrochloride (MXF), in pure forms and pharmaceutical formulations. This method is based on the formation of ion-pair complex between the basic drug (MXF), and acid dye; bromocresol green (BCG). The formed complex was measured at 430 nm by using chloroform as solvent. The analytical parameters and their effects are investigated. Beer's law was obeyed in the range of 0.803 – 12.846 µg/mL, with correlation coefficient R^2 *= 0.9999. The average recovery of Moxifloxacin was between 98.35 and 102.80%. The limit of detection was 9.3 ng/mL and limit of quantification was 27.9 ng/mL. The proposed method has been successfully applied to the analysis of the studied drugs in pure forms and pharmaceutical formulations.*

Keywords: *fluoroquinolone; Moxifloxacin; bromocresol green; Spectrophotometer; pharmaceutical formulations.*

1. INTRODUCTION

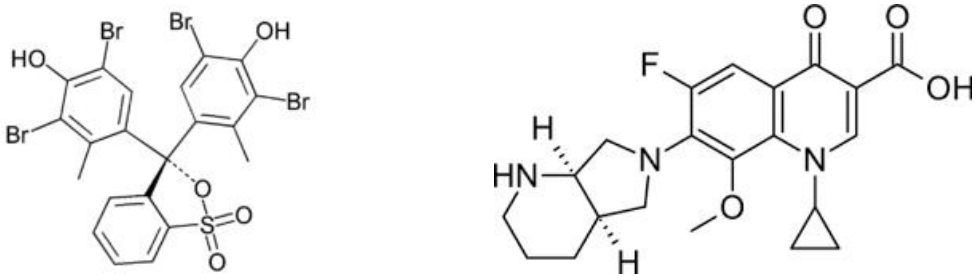
Fluoroquinolones (FQs) are among the most important antibacterial agents used in human medicine. They are active against both Gram-positive and Gram- negative bacteria through inhibition of their DNA gyrase and also possess some activity against mycobacteria, mycoplasmas and rickettsias [1].

Moxifloxacin is a new advanced synthetic generation of fluoroquinolone derivative. Moxifloxacin is active against broad spectrum of pathogens, encompassing Gram-negative, Gram-positive bacteria including resistant strain Streptococcus pneumonia [2,3].

Moxifloxacin, 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3 quinoline carboxylic acid [4].

A wide range of analytical methods have been reported for the determination of moxifloxacin such as, microbiological assay [5], electroanalytical [6,7], protonation equilibrium [8], polarography analytical [9], Capillary electrophoresis [10], High performance thin layer chromatography HPTLC [11-13], Liquid chromatography mass spectrometry (LC/MS)[14], High performance liquid chromatography [HPLC] [15-18], Reverse phase high performance liquid chromatography [RP-HPLC] [19-22], Spectrofluorimetry [23,24], and spectrophotometric methods [25-30].

In the present work, we report the development of accurate and sensitive spectrophotometric method based on the chloroform-soluble ion-pair complexes between the studied fluoroquinolone antibiotic MXF and an acid dye BCG. (Fig. 1). The absorbance measurements were measured at optimum wavelengths. The proposed method was successfully applied for the determination of the studied drugs in pure and pharmaceutical formulations. No interference by the additives was observed. This method is more rapid, economic and sensitive in compared with the previously reported spectrophotometric methods, and it was validated by the statistical data.



Bromocresol green (BCG) Moxifloxacin (MXF)

Fig. 1: The chemical structure of the studied compounds.

2. MATERIALS AND METHODS

2.1 Apparatus

UV/VIS double beam spectrophotometer V-630 (JASCO, Japan) equipped with 1 cm quartz cells; ultrasonic bath (Daihan, Korea); analytical balance TE64 (Sartorius, Germany) with accuracy ± 0.1 mg; digital pipettes (Accumax); drying oven (WTB binder-78532 TUTTLINGEN, Germany).

2.2 Reagents and Materials

All reagents and chemicals used were of analytical reagent or pharmaceutical grade.

Methanol and chloroform (≥99.0%, Sigma-Aldrich).

Bromocresol green (BCG) (≥99.8%, Mw=698.01 $g.mol^{-1,}$ HOPKIN AND WILLIAMS).

Moxifloxacin hydrochloride (MXF) (≥99.2%, Mw=437.89 $g.mol^{-1,}$ ROCHE PHARMA AG, Germany).

Syrian commercial dosage forms of MXF:

- Moxicin tablet (Ibn Al haytham) 400 mg of MXF.
- Moxiflox tablet (Razi) 400 mg of MXF.
- Moxaquin tablet (Obari) 400 mg of MXF.
- Megamox eye drope (Rama pharma), 5 mg of MXF/mL.

2.3 Preparation of solutions.

2.3.1 . Preparation of Standard Stock Solution MXF (2.10^{-3} M)

Standard Stock solution of Moxifloxacin was prepared by dissolving an accurately weighted 88.3 mg of MXF.HCL (including the purity, 99.2 %) in 100mL of Methanol to get a suitable concentration. This solution was prepared fresh daily.

2.3.2 Preparation of Standard Stock Solution BCG (2.10^{-3} M)

Standard Stock solutions of BCG was prepared by dissolving an accurately weighed 139.9 mg (including the purity, 99.8 %) in 100mL of Methanol to get a suitable concentration. This solution are stable for at least one week if kept in the refrigerator.

2.3.3 . Preparation of calibration curve

To prepare calibration standards, compatible volumes of stock standard solutions were diluted with chloroform into volumetric flasks (10 mL) to obtain final drug concentration of (0.803, 1.606, 3.211, 4.817, 6.423, 8.029, 9.634, 11.24, 12.403, 12.846) µg/mL and linearity was studied. Linearity relationship was observed in the range 0.803 to 12.846 µg / mL (Fig. 2) against a reagent blank as reference at 430 nm (Table 1).

3. Estimation of Moxifloxacin in commercial formulations.

3.1 Estimation of Moxifloxacin in tablets.

For analysis of commercial formulations, ten tablets were weighed, powdered and then mixed well. Tablet powder equivalent to 400 mg of Formulation were transferred into 100 ml volumetric flask and dissolved in methanol. Then the solution was sonicated using ultrasonic for 30 minutes and filtered. 1 ml of the filtrate was taken and further diluted with methanol to 100 ml (to form 40 µg/mL). Then 1.25 ml of the last solution was diluted with chloroform to 10 ml (to form 5 µg/mL). The absorbance of the prepared solutions were measured at 430 nm for Moxifloxacin solutions against blank and the drug content was estimated. The results are shown in table (3).

3.2 . Estimation of Moxifloxacin in eye drops.

0.1 ml was taken from eye drop of the mixed contents of five eye drops and diluted with methanol to 10 ml (to form 50 µg/mL). Then 1 ml of the last solution was diluted with chloroform to 10 ml (to form 5 µg/mL). Table (3).

4. RESULTS AND DISCUSSIONS

4.1 Spectrophotometric analysis of Moxifloxacin with BCG.

The method is based on the formation of a yellow ion-pair complex in chloroform, between the fluorquinolone MXF and the reagent BCG. The absorption spectra of the ion-pair complex, MXF-BCG, was measured in the range of 370– 600 nm against the blank solution. The complex has a maximum absorbance at 430 nm as shown in (Fig. 2).

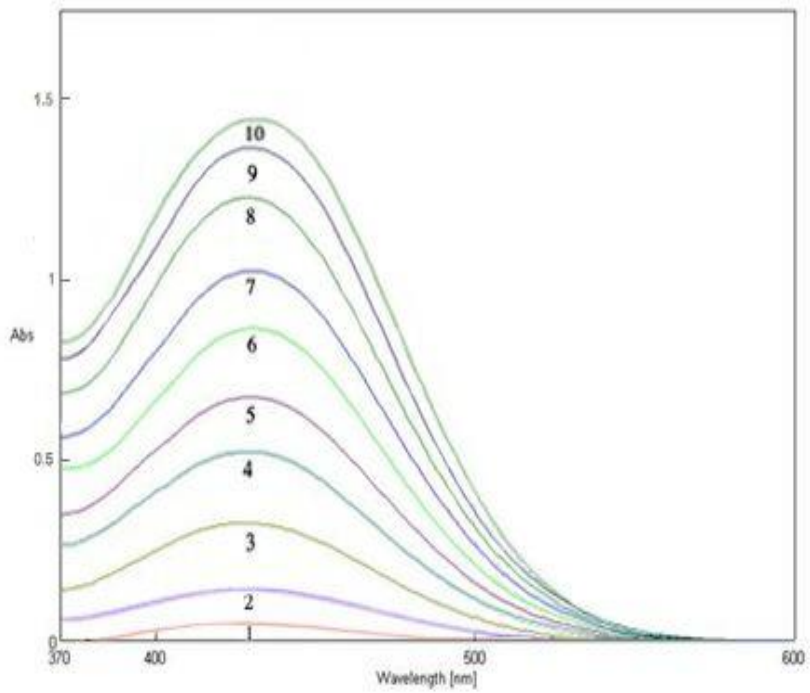


Fig.2: Spectra of complex MXF-BCG for various concentrations of MXF (µg/mL) 1) 0.803, 2) 1.606, 3) 3.211, 4) 4.817, 5) 6.423, 6) 8.029, 7) 9.634, 8) 11.240, 9) 12.403, 10) 12.846

4.2 Optimization of the reaction conditions.

The optimization conditions of the method were carefully studied to achieve complete reaction formation, highest sensitivity, and maximum absorbance.

4.2.1 Effect of solvents.

The effect of several solvents, namely, chloroform, acetone, acetonitrile, benzene, diethyl ether, dichloromethane, dichloroethane, and tetrachloromethane, was studied in order to choose the effective solvent for complex which it had a maximum absorbance.

Chloroform was found to be the most suitable solvent for colored ion-pair complex. Experimental results indicated that the complex with total volume 10 mL chloroform, yielding maximum absorbance intensity, stable absorbance for the studied drugs and considerably lower extraction ability for the reagent blank. The optimization of the method was carefully studied to achieve complete reaction formation, highest sensitivity, and maximum absorbance.

4.2.2 Effect of Reagent Concentration.

The effect of the reagent was studied by measuring the absorbance of solutions containing a fixed concentration of MXF, varied amounts of BCG reagent. Maximum color intensity of the complex was achieved with 1.2 mL of 2.0×10^{-3} M of BCG solution, (Fig. 3).

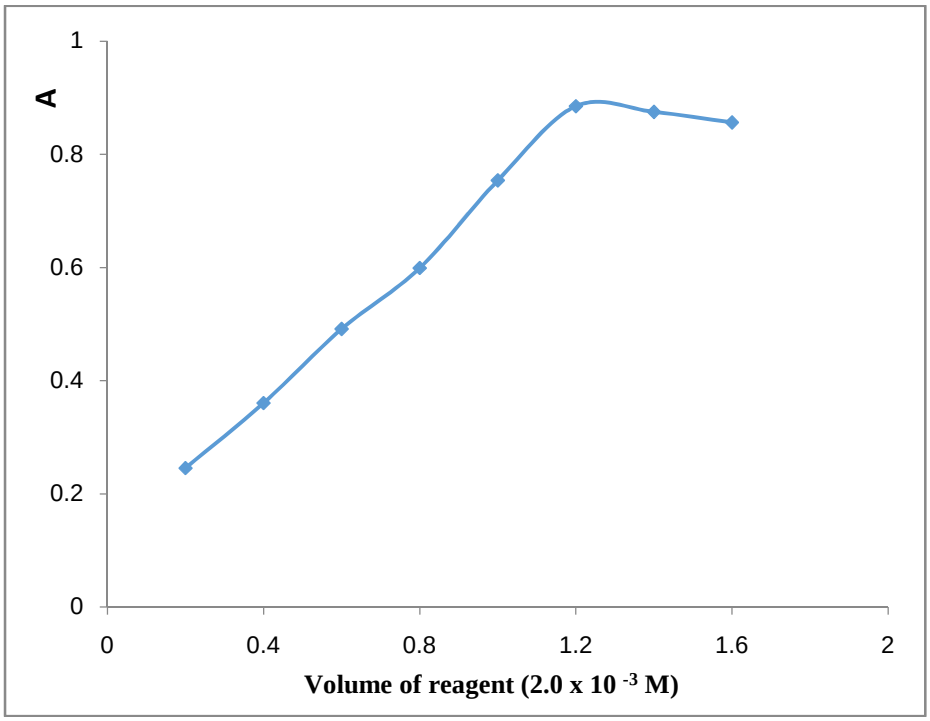


Fig.3: Effect of volume of (2.0 x 10^{-3} M) reagent on the ion-pair complex MXF-BCG

4.2.3 Effect of Time and Temperature.

The optimum reaction time was investigated from 0.5 to 5.0 min by following the colored development at ambient temperature (25 ± 2°C). Complete color intensity was attained after 2.0 min of mixing of the complex. The effect of temperature on colored complexes was investigated by measuring the absorbance values at different temperatures. It was found that the colored complexes were stable up to 35°C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of the chloroform. The absorbance remains stable for at least 10 h at room temperature.

4.3 Stoichiometric Relationship.

The stoichiometric ratio between drug and dye in the complex MXF-BCG was determined by Job's method of the continuous variation method, and Molar ratio method as following:

4.3.1 Job's method of the continuous variation.

Job's method of continuous variation of equimolar solutions was employed: 4.5×10^{-5} M standard solution of MXF and 4.5×10^{-5} M solution of BCG, was used. A series of solutions was prepared in which the total volume of MXF and BCG was kept at 2.0 mL and diluted with chloroform to 10 ml.

A_{max} = f([MXF]/[MXF]+[BCG]), The absorbance was measured at the optimum wavelength. The results showed two molar ratios 1:1 and 2:1 (BCG: MXF),(Fig. 4).

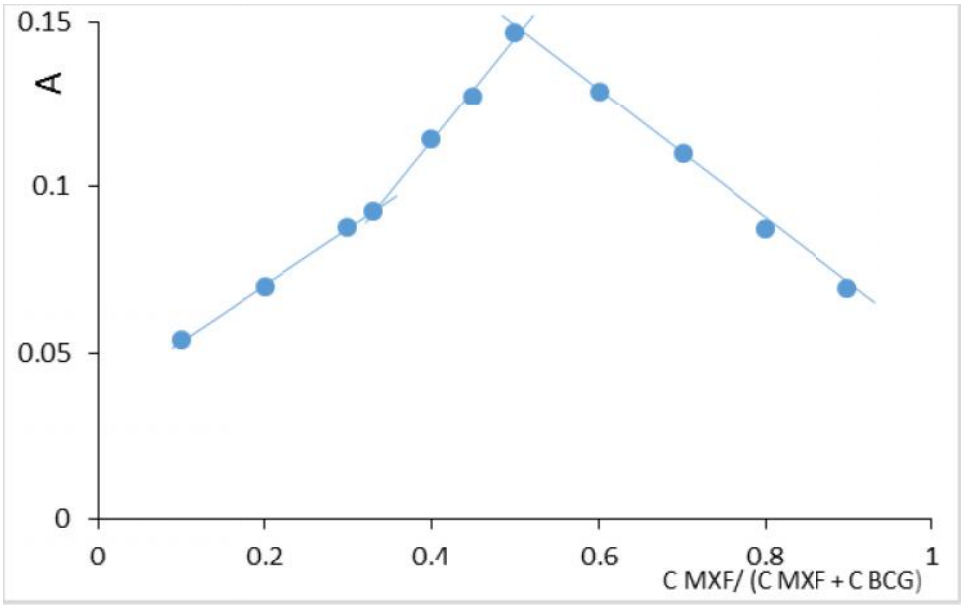


Fig.4. Job's method of the continuous variation of MXF-BCG complex

4.3.2 Molar ratio method.

The stoichiometry of MXF-BCG complex by molar ratio method was studied according to the following equation: A_{max} = f([BCG]/[MXF]), the results confirm the Stoichiometry of complex MXF-BCG that are 1:1 and 2:1 (BCG: MXF); Where the concentration of MXF is constant (4.5×10^{-6} M), and the concentrations of BCG are changed from (0.9 to 12.6) $\times10^{-6}$ M (Fig. 5).

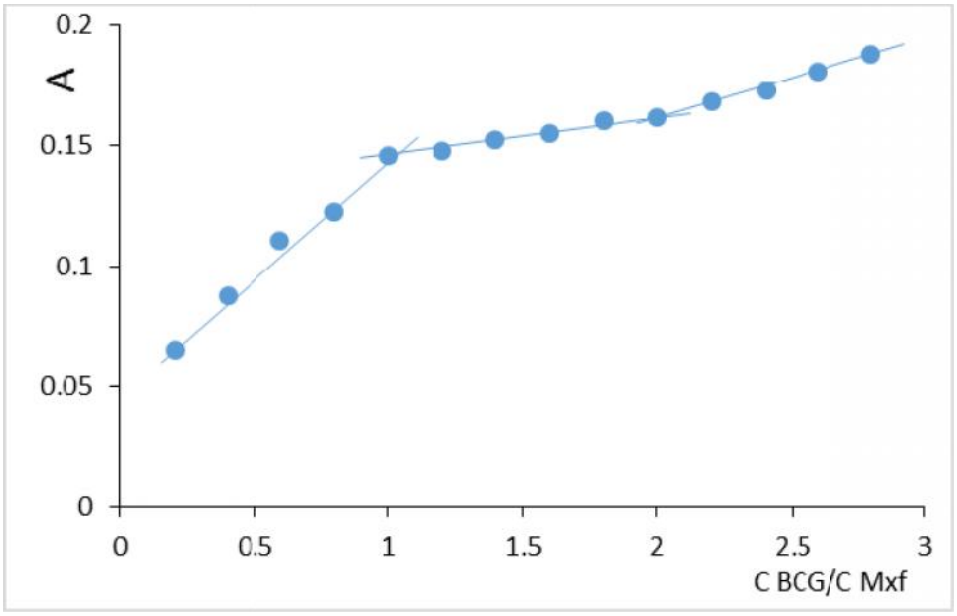


Fig.5. Molar ratio method of MXF-BCG complex

5. METHOD VALIDATION

5.1 The linearity.

At described experimental conditions for MXF determination, standard calibration curves with reagent were constructed by plotting absorbance versus concentration. The absorbance obtained for the five analyses averaged at each concentration. The statistical parameters were given in the regression equation calculated from the calibration graphs. The linearity of calibration graphs was proved by the high values of the correlation coefficient (R^2= 0.9999) and the small values of the *y*-intercepts of the regression equations (fig. 6). The apparent molar absorptivities of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in Table 1.

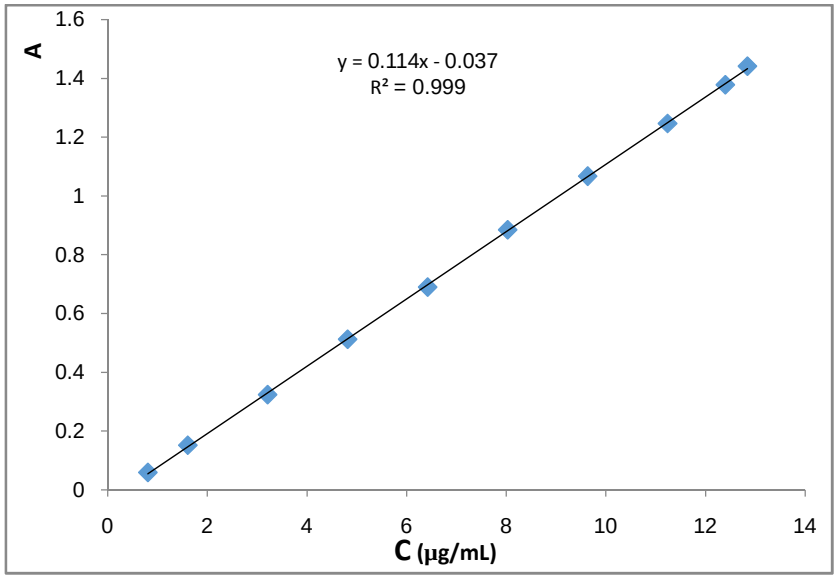


Fig. 6. Calibration curve for determination of MXF – BCG complex

Table 1: Spectrophotometric determination of MXF from MXF-BCG complex in chloroform

(µg/mL)		SD (µg/mL)	RSD%	Analytical standard error ASE $X\frac{SD}{\sqrt{n}}$ (µg/mL)	Confidence Limits $\bar{x} \pm \frac{t \mid SD}{\sqrt{n}}$ (µg/mL)	Recovery %
X taken	X^* found					
0.803	0.823	0.013	1.62	0.006	0.823 ± 0.017	102.49
1.606	1.651	0.025	1.57	0.011	1.651 ± 0.030	102.80
3.211	3.158	0.038	1.18	0.017	3.158 ± 0.047	98.35
4.817	4.802	0.054	1.12	0.024	4.802 ± 0.067	99.69
6.423	6.438	0.063	0.98	0.028	6.438 ± 0.078	100.23
8.029	8.051	0.068	0.85	0.030	8.051 ± 0.083	100.27

9.634	9.647	0.068	0.71	0.030	9.647 ± 0.083	100.13
11.240	11.216	0.079	0.70	0.035	11.216 ± 0.097	99.79
12.403	12.366	0.084	0.68	0.038	12.366 ± 0.105	99.70
12.846	12.919	0.082	0.64	0.037	12.919 ± 0.103	100.57

n = 5

5.2 Sensitivity.

The limits of detection (LOD) and quantitation (LOQ) for the proposed method were found to be 9.3, 27.9 ng/mL respectively, (Table 2).

Table-2 The optimal spectrophotometric parameters for the determination of Moxifloxacin

Parameter	Value
λ_{max} (nm)	430
Beer`s law limits (µg/mL)	0.803 – 12.846
Molar absorptivity, ε (L/mol.cm)	41776.77
Regression equation	Y = 0.1145X - 0.0375
Slope (b)	0.1145
Intercept (C)	- 0.0375
R^2	0.9999
LOD (ng/mL)	9.30
LOQ (ng/mL)	27.9
Sandell sensitivity SS (µg/cm^2)	0.0192

Note: Y = bX+ c, where X is the concentration of drug in µg/mL.

5.3 Accuracy and Precision.

Percentage relative standard deviation (RSD%) and Analytical standard error (ASE%) of the suggested method were calculated and shown in Table 1, These results of accuracy and precision show that the proposed method have good repeatability and reproducibility.

5.4 Analysis of Pharmaceutical Formulations.

The proposed method has been successfully applied to the determination of MXF in pharmaceutical dosage forms in four trade mark Syrian products: Moxicin (Ibn Al haytham), Moxiflox (Razi), Moxaquin (Obari), and Megamox (Rama pharma). Table 3.

Table-3 The obtained results for pharmaceuticals samples

Trade mark	Dose	$\bar{x}^*$ (mg/dose)	RSD%	Recovery %
Moxicin	400 mg/Tab	401.21	1.04	100.30

Moxiflox	400 mg/Tab	396.12	1.04	99.03
Moxaquin	400 mg/Tab	400.91	1.14	100.23
Megamox	5 mg/ml	5.03	1.33	100.60

Moreover, to check the validity of the proposed method, pharmaceutical dosage forms were tested for possible interference with standard addition method (Tables 4,5,6 and 7). There was no significant difference between slopes of calibration curves and standard addition methods. Therefore, it is concluded that the excipients in pharmaceutical dosage forms of MXF did not cause any interference in the analysis of MXF.

Table-4 The recovery of Moxifloxacin in Moxicin

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Moxicin	5	2	6.987	0.83	99.35
	5	4	9.034	0.72	100.85
	5	6	11.042	0.67	100.70

* n = 5

Table-5 The recovery of Moxifloxacin in Moxiflox

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Moxiflox	5	2	7.012	0.78	100.60
	5	4	9.045	0.75	101.12
	5	6	11.056	0.64	100.93

* n = 5

Table-6 The recovery of Moxifloxacin in Moxaquin

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Moxaquin	5	2	7.009	0.88	100.45
	5	4	8.974	0.79	99.35
	5	6	11.068	0.71	101.13

* n = 5

Table-7 The recovery of Moxifloxacin in Megamox

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Megamox	5	2	7.028	0.77	101.40
	5	4	9.021	0.70	100.52
	5	6	11.014	0.64	100.23

* n = 5

6. CONCLUSION

This paper describes the application to form ion-pair complexation reaction with acid dye for the quantification of a fluoroquinolone antibiotic (MXF) in pure forms and pharmaceutical formulations. Compared with the existing visible spectrophotometric methods, the proposed method have the advantages of being relatively simple, rapid, cost-effective, free from auxiliary reagents, and more sensitive for determination of the studied drug in pure form and pharmaceutical formulations. Moreover, the proposed method is free from tedious experimental steps such as heating unlike the previously reported spectrophotometric methods cited earlier. The most attractive feature of this method is their relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal high precision and accuracy of the method besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of the studied drug in pure forms and pharmaceutical formulations.

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