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A Simple and Sensitive Spectrophotometric Method for Determination of Ofloxacin in pure form and Pharmaceutical Formulations

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Abstract: *A Simple, rapid, and sensitive spectrophotometric method was developed for the determination of a fluoroquinolone antibiotic Ofloxacin (OFL), in pure form and pharmaceutical formulations. This method is based on the formation of ion-pair complex between the basic drug (OFL), 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 0.434 – 11.564 µg/mL, with correlation coefficient* R^2 *= 0.9998. The average recovery of Ofloxacin was between 98.61 and 101.61%. The limit of detection was 5.78 ng/mL and limit of quantification was 17.52 ng/mL. The proposed method has been successfully applied to the analysis of the studied drug in pure form and pharmaceutical formulations.*

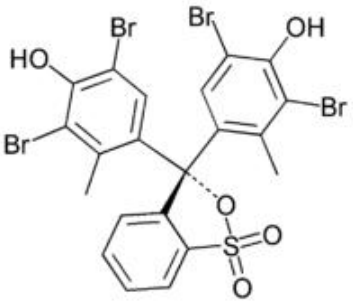
Keywords*: fluoroquinolone; Ofloxacin; bromocresol green; Spectrophotometer; pharmaceutical formulations.*

1. Introduction

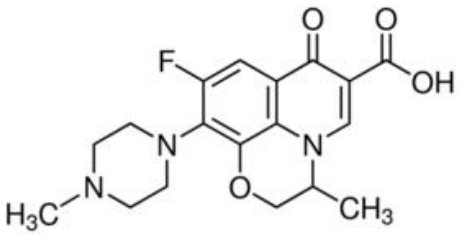
Ofloxacin (OFL) chemically is 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid [1]. It is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. OFL is active against a wide variety of gram-positive and gram negative bacteria. It is used in the treatment of urinary tract infection, conjunctivitis, gonorrhoea, respiratory tract infection and skin infection [2-5].

Several analytical techniques have been used for the determination of ofloxacin as microbiological method [6], capillary electrophoresis [7,8], atomic absorption spectrometry [9], voltammetry [10], potentiometry and conductometry [11], polarography [12], chemiluminescence spectrometry [13–15], HPTLC [16-19], HPLC [20-26], RP-HPLC [27-30], spectrofluorometry [31-33], and spectrophotometry methods [34-41].

In the present work, we report the development of accurate and sensitive spectrophotometric methods based on the chloroform soluble ion-pair complexes between the studied fluoroquinolone antibiotic OFL 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 was observed from the additives. 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)



Ofloxacin (OFL)

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).

Ofloxacin (OFL) (≥99.2%, Mw=361.368 g.mol$^{-1,}$ ROCHE PHARMA AG, Germany).

Syrian commercial dosage forms of OFL:

- Azoflox tablet (Avenzor) 400 mg of OFL.
- Ofloxacin tablet (AL Shahba labs) 400 mg of OFL.
- Ofloxacin eye drops (Delta).

2.3 Preparation of solutions

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

Standard Stock solution of Ofloxacin was prepared by dissolving an accurately weighted 72.8 mg of OFL (including the purity, 99.2 %) in 100mL of Methanol to get a suitable concentration.

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.

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 (0.434, 0.578, 0.723, 1.084, 1.445, 2.891, 4.336, 5.782, 7.227, 8.673, 10.118, 11.564) µg/mL and linearity was studied. Linearity relationship was observed in the range 0.434 to 11.564 µg/mL (Fig. 2) against a reagent blank as reference at 430 nm (Table 1).

3. Estimation of Ofloxacin in commercial formulations

3.1 Estimation of Ofloxacin 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 10 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 Ofloxacin solutions against blank and the drug content was estimated. The results are shown in table (3).

3.2 Estimation of Ofloxacin in eye drops

1 ml was taken from eye drop of the mixed contents of five eye drops and extracted with methanol to 10 ml (to form 300 µg/mL). Then 2 ml of the pervious solution was diluted with chloroform to 10 ml (to form 60 µg/mL). Then 0.83 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 Ofloxacin with BCG

The method is based on the formation of a yellow ion-pair complex in chloroform, between the fluorquinolone OFL and the reagent BCG. The absorption spectra of the ion-pair complex, OFL-BCG, was measured in the range of 350– 600 nm against the blank solution. The complex has a maximum absorbance at 430 nm as shown in (Fig. 2).

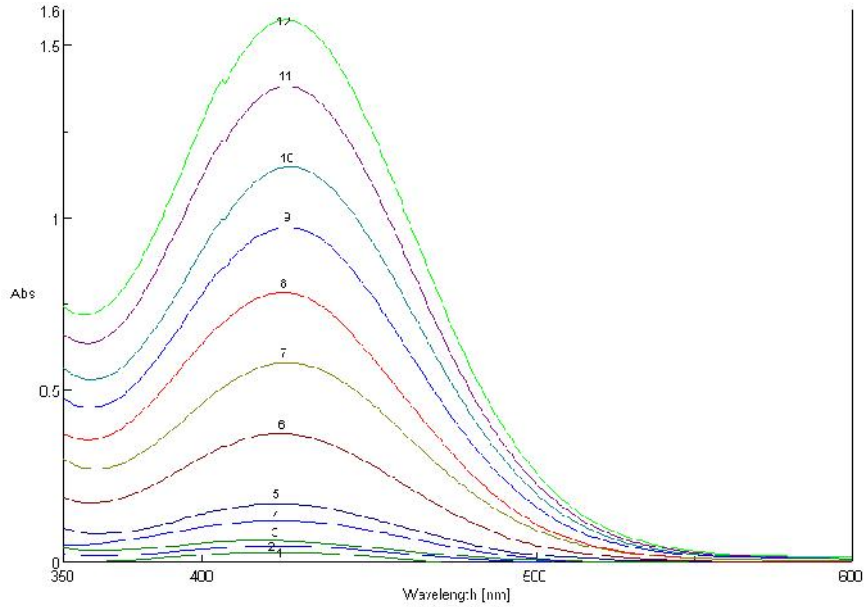


Fig.2: Spectra of complex OFL-BCG for various concentrations of OFL (µg/mL)
1) 0.434, 2) 0.578, 3) 0.723, 4) 1.084, 5) 1.445, 6) 2.891, 7) 4.336,
8) 5.782, 9) 7.227, 10) 8.673, 11) 10.118, 12) 11.564

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 OFL, varied amounts of BCG reagent. Maximum color intensity of the complex was achieved with 1 mL of 2.0×10^{-3} M of BCG solution, (Fig. 3).

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.

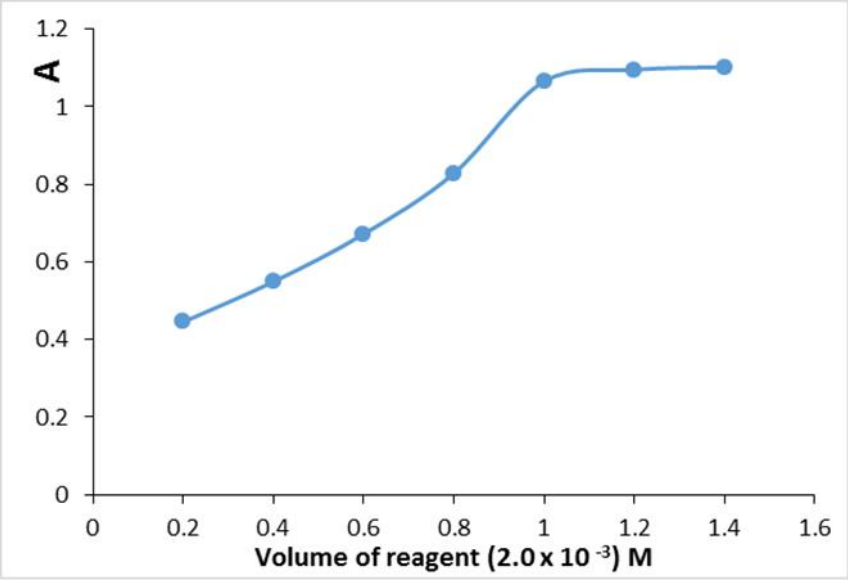


Fig.3: Effect of volume of (2.0 x 10^{-3} M) reagent on the ion-pair complex OFL-BCG

4.3 Stoichiometric Relationship

The stoichiometric ratio between drug and dye in the complex OFL-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: 2 $\times 10^{-4}$ M standard solution of OFL and 2 $\times 10^{-4}$ M solution of BCG, was used. A series of solutions was prepared in which the total volume of OFL and BCG was kept at 2.0 mL and diluted with chloroform to 10 ml.

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

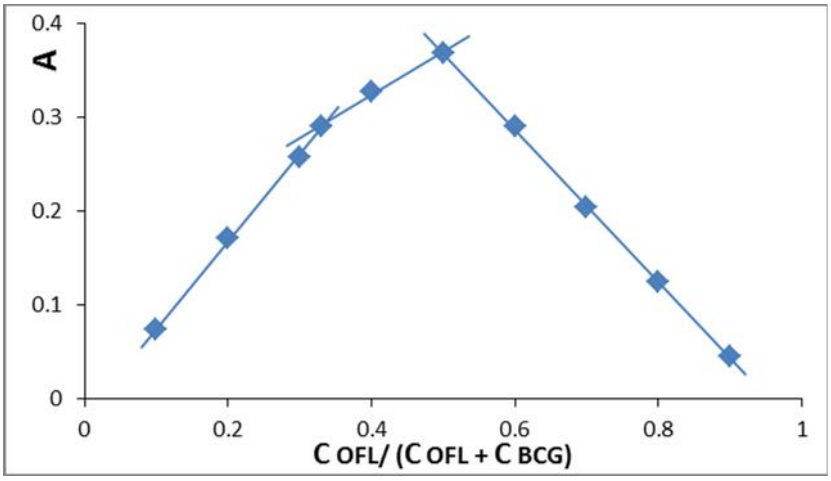


Fig.4: Job's method of the continuous variation of OFL-BCG complex

4.3.2 Molar ratio method

The stoichiometry of OFL-BCG complex by molar ratio method was studied according to the following equation: A_{max} = f([BCG]/[OFL]), the results confirm the Stoichiometry of complex OFL-BCG that are 1:1 and 2:1 (BCG: OFL); Where the concentration of OFL is constant (2 $\times 10^{-5}$ M), and the concentrations of BCG are changed from (0.4 to 5.2) $\times 10^{-5}$ M (Fig. 5).

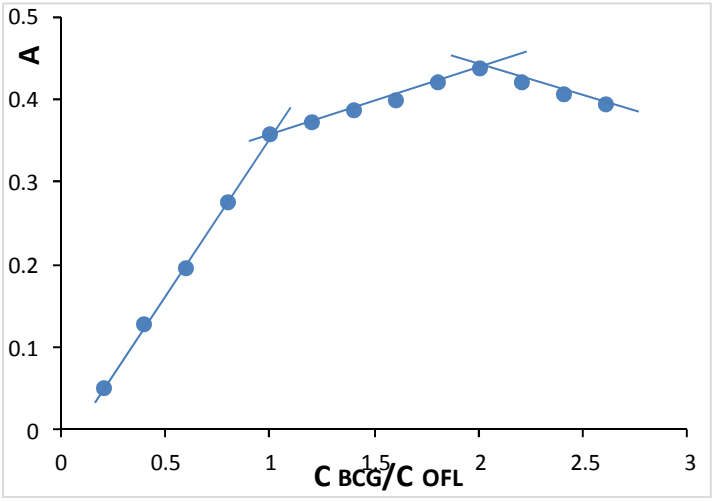


Fig.5: Molar ratio method of OFL-BCG complex

5. Method Validation

5.1 The linearity

At described experimental conditions for OFL determination, standard calibration curves with reagent was 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= 0.9998) 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.

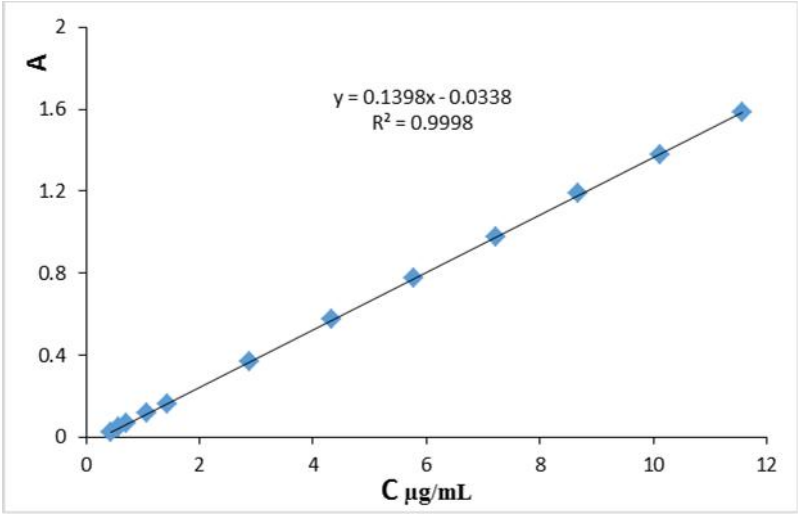


Fig.6: Calibration curve for determination of OFL – BCG complex

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

(µg/mL)		SD (µg/mL)	RSD%	Analytical standard error $ASE\ X\frac{SD}{\sqrt{n}}$ (µg/mL)	Confidence Limits $\bar{x} \left\{ \frac{t \mid SD}{\sqrt{n}} \right.$ (µg/mL)	Recovery %
X taken	X^* found					
0.434	0.441	0.009	2.04	0.0040	0.441 ± 0.0111	101.61
0.578	0.576	0.011	1.91	0.0049	0.576 ± 0.0136	99.65
0.723	0.725	0.012	1.65	0.0054	0.725 ± 0.0150	100.28
1.084	1.101	0.015	1.36	0.0067	1.101 ± 0.0186	101.57

1.445	1.425	0.018	1.26	0.0080	1.425 ± 0.0222	98.61
2.891	2.879	0.032	1.11	0.0143	2.879 ± 0.0397	99.58
4.336	4.379	0.045	1.03	0.0201	4.379 ± 0.0558	100.99
5.782	5.793	0.056	0.97	0.0250	5.793 ± 0.0694	100.19
7.227	7.240	0.064	0.88	0.0286	7.240 ± 0.0794	100.18
8.673	8.735	0.073	0.84	0.0326	8.735 ± 0.0905	100.71
10.118	10.080	0.077	0.76	0.0344	10.080 ± 0.0955	99.62
11.564	11.600	0.078	0.67	0.0349	11.600 ± 0.0969	100.31

* n = 5

5.2 Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed method were found to be 5.78, 17.52 ng/mL respectively, (Table 2).

Table 2: The optimal spectrophotometric parameters for the determination of Ofloxacin

Parameter	Value
λ_{max} (nm)	430
Beer`s law limits (µg/mL)	0.434 – 11.564
Molar absorptivity, ε (L/mol.cm)	42133.57
Regression equation	Y = 0.1398X - 0.0338
Slope (b)	0.1398
Intercept (C)	- 0.0338
R^2	0.9998
LOD (ng/mL)	5.78
LOQ (ng/mL)	17.52
Sandell sensitivity SS (µg/cm^2)	0.0171

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 OXF in pharmaceutical dosage forms in four trade mark Syrian products: Azoflox tablet (Avenzor), Ofloxacin tablet (AL Shahba labs), and Ofloxacin eye drops (Delta), Table 3.

Table 3: The obtained results for pharmaceuticals samples

Trade mark	Dose	$\bar{x}^*$ (mg/dose)	RSD%	Recovery %
Azoflox (tablet)	400 mg/Tab	404.16	0.94	101.04
Ofloxacin (tablet)	400 mg/Tab	399.49	1.04	99.87
Ofloxacin (eye drops)	3 mg/ml	3.01	1.69	100.33

Moreover, to check the validity of the proposed method, pharmaceutical dosage forms were tested for possible interference with standard addition method (Tables 4,5 and 6). 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 OXF did not cause any interference in the analysis of OXF.

Table 4: The recovery of OFL in Azoflox tablet (Avenzor)

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Azoflox tablet	5	2	7.014	0.83	100.`70
	5	4	9.056	0.72	101.40
	5	6	11.058	0.69	100.97

* n = 5

Table 5: The recovery of OFL in Ofloxacin tablet (AL Shahba labs)

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Ofloxacin tablet	5	2	6.987	0.86	99.35
	5	4	8.981	0.75	99.52
	5	6	11.023	0.72	100.38

* n = 5

Table 6: The recovery of OFL in Ofloxacin eye drops (Delta)

Ofloxacin	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %

eye drops	**5**	2	7.018	0.87	100.90
	5	4	8.987	0.73	99.67
	5	6	11.044	0.67	100.73

* 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 (OFL) 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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