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Spectrophotometric determination of Pefloxacin through ion-pair complexin pharmaceuticals

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Abstract :*A Simple, rapid, accurate, and sensitive spectrophotometric method was developed for the determination of Pefloxacin (PEF), in pure forms and pharmaceutical formulations. This method is based on the formation of ion-pair complex between the basic drug (PEF), and acid dye; bromocresol green (BCG). The formed complex was measured at 432 nm by using chloroform as solvent. The analytical parameters and their effects are investigated. Beer's law was obeyed in the rangeof2.000 – 14.668 µg/mL, with correlation coefficient* R^2 *= 0.9999. The average recovery of Pefloxacin was between 98.50and 101.65%. The limit of detection was 17.84ng/mL and limit of quantification was 54.07ng/mL. The proposed method has been successfully applied to the analysis of PEF in pure forms and pharmaceutical formulations.*

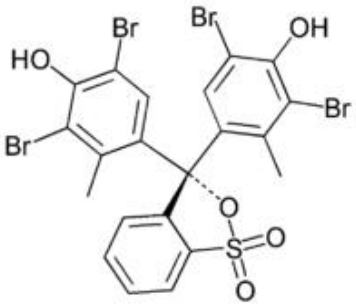
Keywords: *Pefloxacin; bromocresol green; Spectrophotometer; pure forms; pharmaceutical formulations.*

INTRODUCTION

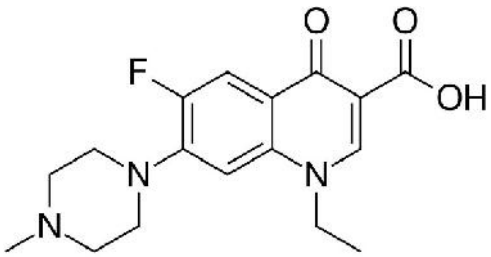
Pefloxacin mesylate is described chemically as 1-ethyl-6-fluoro-7-(4-methylpiperazinyl-1)-4-oxo-1, 4-dihydro quinoline-3-carboxylic acid methane sulphonate was introduced in 1985 as a new chemical entity. It is a broad spectrum third generation fluoroquinolone antibiotic active against both gram positive and gram negative bacteria [1-3]. It is used in the treatment of various respiratory tract infection, urinary tract infection, sexually transmitted diseases and gastrointestinal infection. [4].

Suitable and sensitive analytical methods for determination of drug are essential for successful evaluation in their dosage form. Several analytical methods for quantitative determination of pefloxacin in pharmaceutical formulations and in biological fluids are described in the literature, such as: capillary electrophoresis [5-9], Atomic absorption spectroscopic[10,11], conductometric [12], and colorimetric methods[13,14], TLC-fluorescence [15-17], voltammetry [18],UPLC [19], HPLC [20-27], chemiluminescence spectrometry [28-31], spectrofluorometry [32-39], and spectrophotometry methods [40-48].

In Previous Researches, we developed Simple, rapid and highly sensitive method for determination of Moxifloxacin, and Ofloxacin[49-51].In the present work, we report the development of accurateand sensitive spectrophotometric methodbased on the chloroform soluble ion-pair complexes between the studied fluoroquinolone antibiotic PEF 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 morerapid, economic andsensitivein compared withthe previously reported spectrophotometric methods, anditwas validated by the statistical data.



Bromocresol green (BCG)



Pefloxacin (PFF)

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

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).

Pefloxacinm esylate (PEF) (≥94.6%, Mw=429.463 g.mol^{-1},ROCHE PHARMA AG, Germany).

Syrian commercial dosage forms of PEF:

- Peflacine tablet (Oubaripharma) 400 mg of PEF.
- Peflox tablet (Racha) 400 mg of PEF.

2.3 Preparation of solutions

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

Standard Stock solution of Pefloxacin was prepared by dissolving an accurately weighted 90.8mg of Pefloxacin mesylate(including the purity, 94.6 %) 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 Preparationof calibration curve

To prepare calibration standards,compatible volumes ofstock standard solutionswere dilutedwith chloroform into volumetric flasks (10 mL) to obtain final drug concentration of, (2.000, 2.667, 4.000, 5.334, 6.667, 8.000, 9.334, 10.667, 12.001, 13.334, 14.677)µg/mL and linearity was studied. Linearityrelationship was observed in the range 2.000to 14.677 µg / mL (Fig. 2) against a reagent blank as reference at 432 nm (Table 1).

Estimation of Pefloxacinin commercial formulations

3.1 Estimation of Pefloxacin in tablets

For analysis of commercial formulations, ten tablets were weighed, powdered andthenmixed 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 ofthe last solution was diluted with chloroform to 10 ml (to form 5 µg/mL).

The absorbance of the prepared solutions were measured at 432 nm for Pefloxacin solutions against blank and the drug content was estimated. The results are shown in table(3).

RESULTS AND DISCUSSIONS

4.1 Spectrophotometric analysis of Pefloxacin with BCG

The method is based on the formation of a yellow ion-pair complex in chloroform, between the fluorquinolone PEF and the reagent BCG. The absorption spectra of the ion-pair complex, PEF-BCG, was measure dintherange of 350– 600nm against the blank solution. The complex has a maximum absorbance at 432 nm as shown in (Fig. 2).

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, dichlorom ethane, dichloro ethane, and tetrachloro methane, was studied in order to choose the effective solvent for complex which it had a maximum absorbance.

Chloroform was found to bethemostsuitablesolventforcolored ion-pair complex. Experimental results indicated that the complex withtotalvolume10mL chloroform, yielding maximum absorbance intensity, stable absorbance for the studied drugs and considerably lower extractionabilityforthereagentblank. The optimization of the method was carefully studied to achievecompletereactionformation,highestsensitivity,and maximum absorbance.

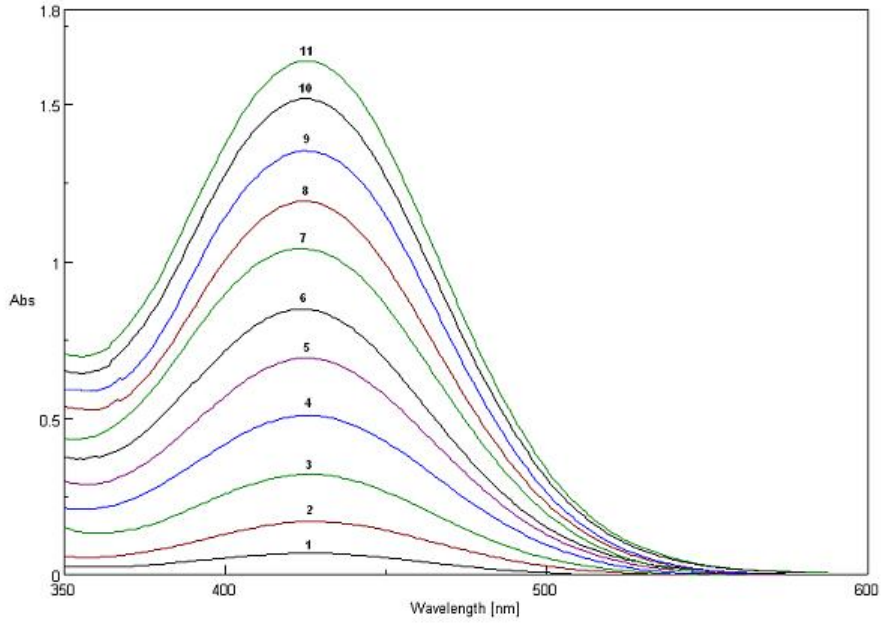


Fig.2: Spectra of complexPEF-BCG for various concentrations of PEF (µg/mL) 1) 2.000, 2) 2.667, 3) 4.000, 4) 5.334, 5) 6.667,6) 8.000, 7) 9.334, 8) 10.667, 9) 12.001, 10) 13.334, 11) 14.677

4.2.2 Effect of Reagent Concentration

The effect of the reagent Concentration was studied by measuring the absorbance of solutions containing a fixed concentration of Pef (2.0×10^{-4} M), varied amounts of BCG reagent (2.0×10^{-3} M). The result shows that 1mL of 2.0×10^{-3}Mof BCG solution found to be optimum for this proposed method.

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 StoichiometricRelationship

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

A_{max}= f([PEF]/[PEF]+[BCG]), The absorbance was measured at the optimum wavelength. The results showed two molar ratios 1:1 and 2:1 (BCG: PEF),(Fig. 3).

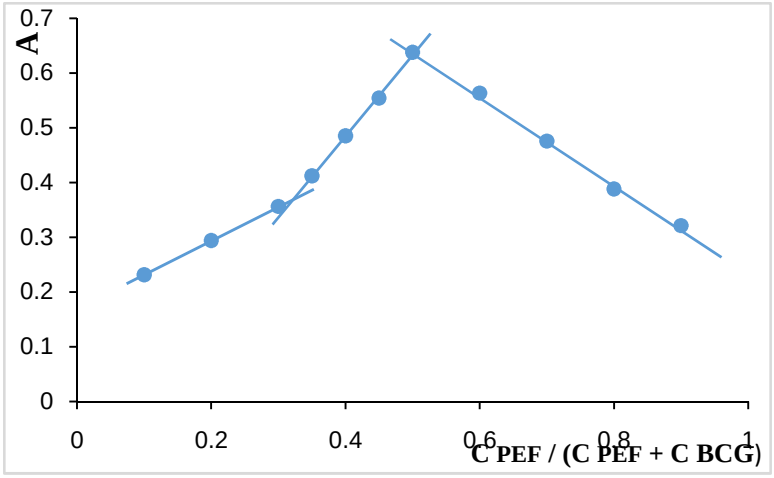


Fig.3: Job's method of the continuous variation of PEF-BCG complex

4.3.2Molar ratio method

The stoichiometry of PEF-BCG complex by molar ratio method was studied according tothefollowing equation: A_{max}= f([BCG]/[PEF]), the results confirm the Stoichiometry of complex PEF-BCG that are 1:1 and 2:1 (BCG: PEF); Where the concentration of PEF is constant (2 $\times 10^{-5}$ M), and the concentrations of BCG are changed from (0.4 to 5.6) $\times 10^{-5}$ M(Fig. 4).

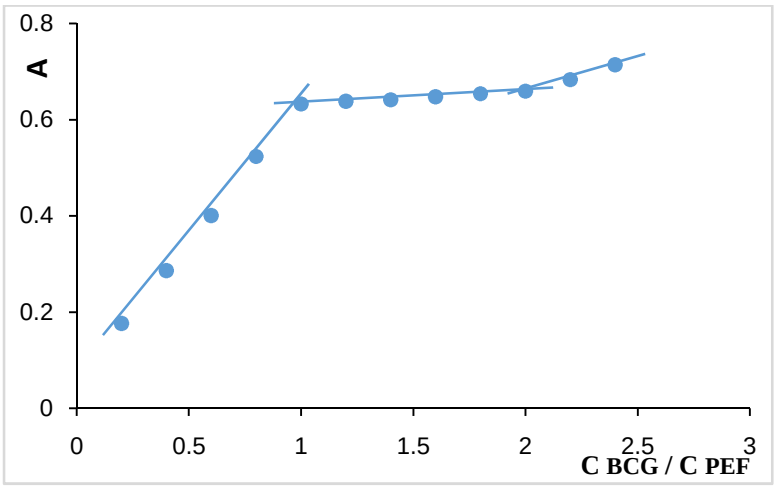


Fig.4: Molar ratio method of PEF-BCG complex

METHOD VALIDATION

5.1 The linearity

At described experimental conditions for PEF determination, standard calibration curves with reagen was constructed by plotting absorbance versus concentration .The absorbance obtained for the five analyses averaged at each concentration. Thestatisticalparameterswereg ivenin 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. 5).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 Table1.

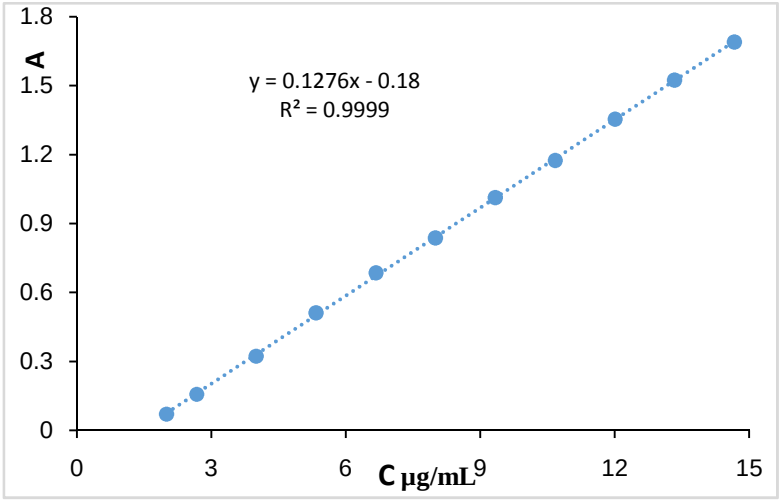


Fig. 5:Calibration curve for determination of PEF – BCG complex

Table 1: Spectrophotometric determination of PEF from PEF-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					
2.000	1.970	0.039	1.98	0.0174	1.970 ± 0.0483	98.50
2.667	2.646	0.048	1.81	0.0215	2.646 ± 0.0597	99.21
4.000	3.947	0.065	1.65	0.0291	3.947 ± 0.0808	98.67
5.334	5.418	0.077	1.42	0.0344	5.418 ± 0.0955	101.57
6.667	6.777	0.086	1.27	0.0385	6.777 ± 0.1069	101.65
8.000	7.968	0.091	1.14	0.0407	7.968 ± 0.1130	99.60
9.334	9.342	0.097	1.04	0.0434	9.342 ± 0.1205	100.09
10.667	10.616	0.102	0.96	0.0456	10.616 ± 0.1266	99.52
12.001	12.023	0.107	0.89	0.0478	12.023 ± 0.1327	100.18
13.334	13.351	0.104	0.78	0.0465	13.351 ± 0.1291	100.13
14.668	14.650	0.109	0.74	0.0487	14.650 ± 0.1352	99.88

n = 5

5.2 Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed method were found to be 17.84, 54.07ng/mL respectively, (Table 2).

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

Parameter	Value
λ_{max} (nm)	432
Beer`s law limits (µg/mL)	2.000 – 14.668
Molar absorptivity, ε (L/mol.cm)	31507.72
Regression equation	Y = 0.1276X –0.18
Slope (b)	0.1276
Intercept (C)	- 0.18
R^2	0.9999
LOD (ng/mL)	17.84
LOQ (ng/mL)	54.07
Sandell sensitivity SS (µg/cm^2)	0.0212

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 methodhas been successfully applied to the determination of PEF in pharmaceutical dosage forms in four trade mark Syrian products: Peflacine(Oubaripharma), Peflox(Racha)Table 3.

Table 3:The obtained results for pharmaceuticals samples

Trade mark	Dose	$\bar{x}^*$ (mg/dose)	RSD%	Recovery %
Peflacine	400 mg/Tab	398.06	0.91	99.51
Peflox	400 mg/Tab	403.13	1.01	100.78

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

Table 4:The recovery of PEF in Peflacine(Oubaripharma)

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Peflacine	5	2	6.983	0.73	99.15

	5	4	8.987	0.59	99.67
	5	6	11.058	0.50	100.97

* n = 5

Table 5:The recovery of PEF in Peflox(Racha)

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Peflox	5	2	7.012	0.72	100.60
	5	4	9.041	0.62	101.25
	5	6	10.981	0.55	99.68

* n = 5

CONCLUSION

The proposed UV spectrophotometric method for the estimation of Pefloxacin Mesylate is simple, sensitive and economical. This method was also validated by checking the parameterssuch as accuracy, precision, linearity, robustness and ruggedness. The proposed method showed high level of precision as depicted by low values of standard deviation and relativestandard deviation. Hence this method can be used routinely for rapid assay of Pefloxacin in bulk and pharmaceutical formulations without interference of excipients andother additives.

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