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Simple and sensitive method for the spectrophotometric determination of pefloxacin

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ABSTRACT

Pefloxacin (PEF) is a well-known fluoroquinolone effective against both Gram-positive and Gram-negative bacteria. Due to its great therapeutic importance, simple method has been proposed for the spetrophotometric determination of this drug. Ceric ammonium sulphate (CAS) is used for the oxidation of PEF and the excess oxidant is determined by using either xylene cyanol FF (XCFFF) or safranin O (SO). The calibration graphs are found to be linear over 15.00–35.00 and 10.00–60.00 μg mL^{-1} with molar absorptivity values of 2.05 × 10^4 and 2.95 × 10^4 L $mol^{-1}cm^{-1}$ for PEF-XCFF and PEF-SO, respectively. The methods have been successfully applied for the determination of drugs in pharmaceutical dosage forms.

Keywords: Pefloxacin, spectrophotometry, ceric ammonium sulphate, xylene cyanolff, safranin O

INTRODUCTION

Pefloxacin (PEF), chemically known as1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid (**Figure 1**),is one of the latest broad-spectrum antibacterial 4-quinolones. It is water soluble, with a pH range of 3 to 4.5 in aqueous solution. PEF is the member of second generation of the quinolone group antibiotics. It acts by inhibiting the bacterial enzyme DNA gyrase which is responsible for supercoiling of DNA. Due to its intensive and efficient antibacterial activity comparing with the common quinolones, it has been widely used in clinical practice. PEF is given by mouth or by intravenous infusion in the treatment of susceptible infections. It is well absorbed by the oral route. Its bio-availability is 100 % and it has a long plasma half-life [1-4].The therapeutic magnitude of PEF has necessitated the progress of analytical methods for its determination in dosage forms. Several analytical procedures have been established for the determination of PEF. These comprise HPLC [5-11], spectrofluorimetry [12], spectrophotometry[13-19],chemiluminescence [20].

All these reported methods are either not adequately sensitive or tiresome and require highly sophisticated instrumentation. Hence a new spectrophotometric method was developed to determine PEF in pure as well as dosage form. A comparison for the performance characteristics of the proposed methodwith the reported methods is given in **Table 1**.The present investigation deals with the oxidation of PEF by CAS. The residual oxidant is determined by using either XCFF or SO measuring the absorbances at 612 and 519 nm, respectively. The proposed method has been applied successfully for the determination of PEF in pharmaceutical formulation.

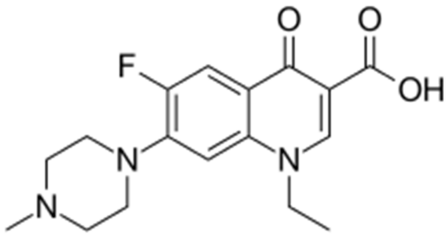

Figure 1:Pefloxacin

Table 1: A comparison table for the performance characteristics of the proposed method with the reported methods

Reagent	Range (µg mL^{-1})	λmax (nm)	Molar absorptivity (L mol^{-1} cm^{-1})	Reference
NaOH-Phenol red	5.00–40.00	560	5.91×10^3	[17]
Folin-Ciocalteau Reagent	10.00–45.00	670	2.80×10^3	[18]
Iron(III) Nitrate - $NaNO_3$	4.70–13.70	404	4.80×10^3	[19]
Proposed method				
CAS – XCFF	15.00-35.00	612	2.05×10^4	----
CAS – SO	10.00-60.00	519	2.95×10^4	----

MATERIALS AND METHODS

2.1. Apparatus

A UV-Visible spectrophotometer (SHIMADZU, Model No.UV-2550) with 1 cm quartz cells was used for all the measurements.

2.2. Reagents and Solutions

All solutions were prepared with distilled water. A 1000 µg mL^{-1}PEFsolution was prepared in distilled water. All the chemicals used were of analytical grade. Solutions of ceric ammonium sulphate (CAS) (0.4 %), H_2SO_4 (2 M), xylene cyanol FF (XCFF) (0.1 %) and safranin O (SO) (0.025 %) were prepared. CAS was standardized with Mohr's salt by using ferroin as indicator.

2.3. Procedures

2.3.1. Determination of PEF using CAS - XCFF as reagents

Different aliquots of PEF containing 15.00 - 35.00 µg mL^{-1} were transferred into a series of 10 mL standard flasks with the help of a micro burette. Then 1.0 mL of 0.4 % CAS was added to each flask, shaken well and kept for 5 min for the reaction to complete. Then 0.8 mL of 0.1 % XCFF was added to each flask and shaken well. The reaction mixture was diluted to 10 mL with distilled water. The absorbance of each solution was measured at 612 nm.

2.3.2. Determination of PEF using CAS - SO as reagents

Different aliquots of PEF containing 10.00 - 60.00 µg mL^{-1} were transferred into a series of 10 mL standard flasks with the help of a micro burette. Then 1.0 mL of 0.4 % CAS was added to each flask, shaken well and kept for 5 min for the reaction to complete. Then 1.5 mL of 0.025 % SO was added to each flask and shaken well. The reaction mixture was diluted to 10 mL with distilled water. The absorbance of each solution was measured at 519 nm.

2.3.3. Assay of formulation

The proposed method was applied to the determination of PEF in a representative tablet (Pelox). Tablet weight equivalent of 400 mg of PEFwas taken and ground using a mortar and pestle. The sample stock solution was prepared by dissolving the powder in ethanol, shaken for 30 min and filtered through Whatman No.41 filter paper. Clear solution was made up to 100 mL. Known amount of the solution was taken and analyzed for PEF following the above procedure without any modification.

RESULTS AND DISCUSSION

2.4.1. Absorption Spectra and Optimization of Reagent Concentrations

Among various other oxidizing agents, CAS isutilized extensively for the determination of organic compounds. The proposed procedure involves two steps; the first is concerned with the treatment of the investigated drug with a known excess amount of CAS in 2 M H_2SO_4 for certain time. The second step involves the determination of the excess unreacted CAS *via* its reaction with either XCFF or SO. The method involves the oxidation of PEF by CAS and the surplus oxidant is determined by using either XCFF or SO. The excess of CAS bleaches the dyes XCFF and SO. The increase in the absorbance with the concentration of the drug is measured at 612 and 519 nm for PEF -

XCFF and PEF - SO systems, respectively. Preliminary experiments show that 1.0 mL of 0.4 % CAS is sufficient to oxidize the drug. The adherence to Beer's law and absorption spectra of the systems are given in **Figures (2-5)**.

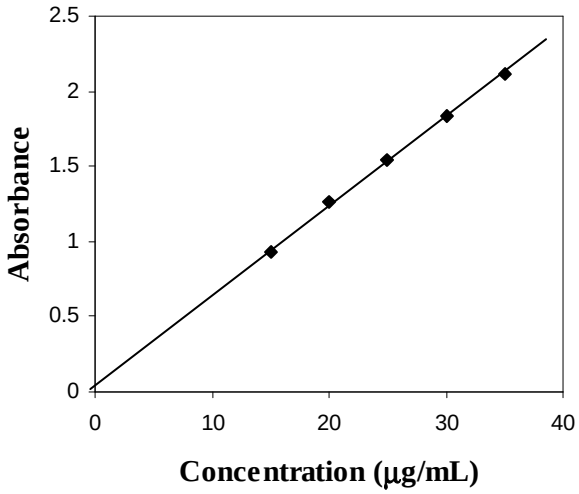

Figure 2: Adherence of Beer's law for spectrophotometric determination of PEF using CAS - XCFF as reagents

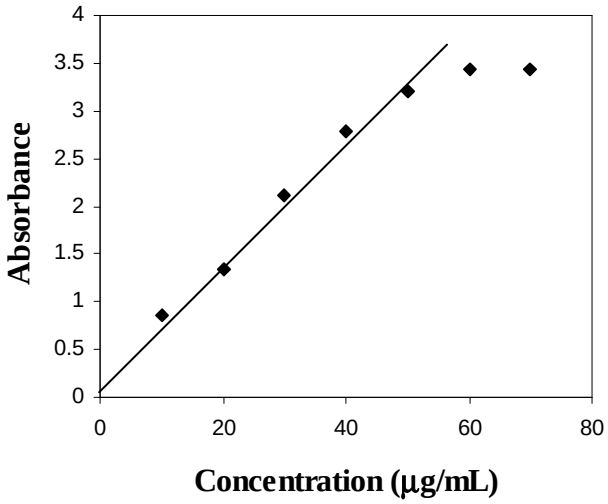

Figure 3: Adherence of Beer's law for spectrophotometric determination of PEF using CAS - SO as reagents

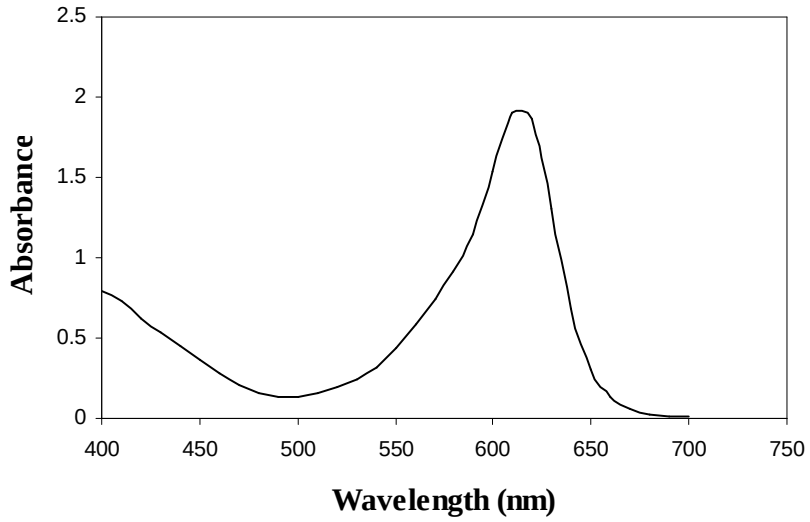

Figure 4: Absorption spectrum of PEF - XCFF system

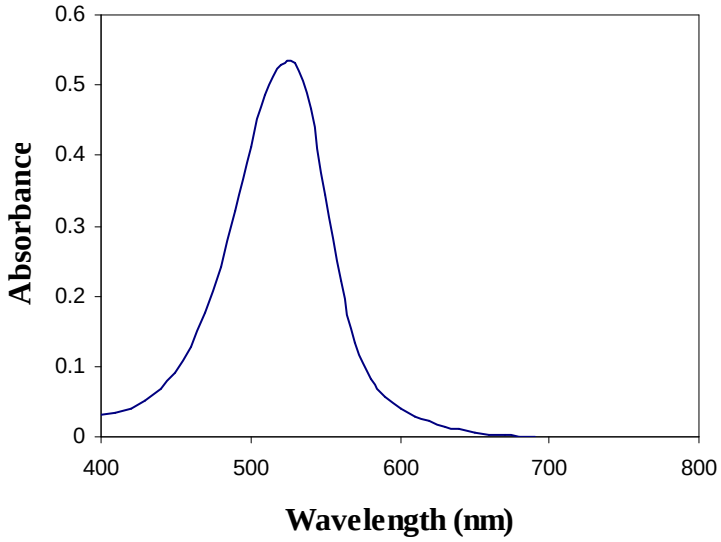

Figure 5: Absorption spectrum of PEF- SO system

The optimum conditions for the development of method are established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored product. The drug undergoes oxidation and is found to be complete in 5 min. The reaction is carried out in sulphuric acid medium. A volume of 1 mL of 2 M sulphuric acid is used in the reaction, as this concentration is found to be ideal.

2.4.2. Analytical Data

Under the optimum reaction conditions described above, the calibration curves for PEF using the proposed method are constructed. In both cases, Beer's law plots are linear with very small intercepts and good correlation coefficients in the general concentration range of 15.00 – 35.00 and 10.00 – 50.00 μg mL^{-1} for PEF - XCFF and PEF - SO, respectively **(Table 2)**. Statistical analysis of the results obtained, indicated that the proposed methods are accurate and precise. The limit of detection (LOD) and limit of quantitation (LOQ) calculated according to ICH (International Conference on Harmonization) guidelines [21]as LOD =3.3 × σ/S and LOQ = 10 × σ/S, where σ is standard deviation of y-intercept of regression lines (standard deviation of response) and S is slope of calibration curve. Sensitivity of proposed methods are determined by calculating Sandell's sensitivity (μg cm^{-2} per 0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross section. The calibration graphs are described by the equation Y = a + bX (Y = absorbance, a = intercept, b = slope, X = concentration in μg mL^{-1}) obtained by the method of least squares.

Table 2: Analytical parameters

	PEF – XCFF	PEF - SO
λmax (nm)	612	519
Beer's Law Limit (µg mL^{-1})	15.00-35.00	10.00-60.00
Molar Absorptivity (L mol^{-1} cm^{-1})	2.05×10^4	2.95×10^4
Limit of Detection** (µg mL^{-1})	1.0820	0.5365
Limit of Quantification** (µg mL^{-1})	3.2890	1.6260
Regression Equation*	Y = a + bX	Y = a + bX
Slope (b)	0.0585	0.0615
Intercept (a)	0.0744	0.2127
Correlation Coefficient (r)	0.9995	0.9955

**Y is the absorbance and X is the concentration in (µg mL^{-1}).*
*** Calculated using ICH-Guidelines.*

2.4.3. Method Validation

The accuracy of the method is established by analyzing the pure drug at different levels within working limits and the precision is ascertained by calculating the relative standard deviation of replicate determinations on the same solution containing the drug at different levels and are presented in **Table 3**& **4.**The relative error and relative standard deviation indicate the high accuracy and precision for the method. In order to check the validity of the proposed methods, PEF is determined in commercial formulation. From the results it is clear that there is close agreement between the result obtained by the proposed method and the label claim.

Table 3: Evaluation of accuracy and precision (Using CAS - XCFF as reagents)

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
15.00	14.98	0.13	0.03	0.20
20.00	20.01	-0.05	0.04	0.19
25.00	25.02	-0.08	0.04	0.16
30.00	29.98	0.06	0.04	0.13

**Mean value of five determinations.*
RE - Relative Error, SD - Standard Deviation; RSD - Relative Standard Deviation.

Table 4: Evaluation of accuracy and precision(Using CAS – SO as reagents)

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
10.00	9.99	0.10	0.019	0.19
20.00	19.98	0.10	0.016	0.08
30.00	29.98	0.06	0.026	0.06
40.00	39.96	0.10	0.023	0.05
50.00	49.97	0.06	0.023	0.04

**Mean value of five determinations.*
RE - Relative Error, SD - Standard Deviation; RSD - Relative Standard Deviation.

2.4.4. Applications

The proposed method has been successfully applied to the determination of PEF in dietary supplement. The content of the tablet formulation is calculated by applying suitable dilution factor. The results are compared statistically with those of the tabulated value at 95 % confidence level. The calculated student's t-test does not exceed the tabulated value, indicating that there is no significant difference between the proposed method and the tabulated value in respect to accuracy and precision **Table 5**.

Table 5: Results of assay of formulations.

Brand name	Pelox[a]
Labeled amount (mg)	400.0
1. PEF - XCFF	
i. Amount found* (mg)	399.6
ii. % Label claim ± SD	99.90 ± 0.08
iii. t-test	1.069
2. PEF - SO	
i. Amount found* (mg)	399.0
ii. % Label claim ± SD	99.75 ± 0.03
iii. t-test	1.069

[a]Wockhardt Ltd., India
**Mean value of five determinations.*
Value of 't' at 95 % confidence level = 3.182.
Value of 'F' at 95 % confidence level = 6.39.

CONCLUSION

Simple spectrophotometric method for the determination of PEF has been developed. The proposed method is sensitive, accurate and precise and has low RSD values. All the analytical reagents are inexpensive, have excellent shelf life and are available in any analytical laboratory. The proposed method is advantageous when compared to many of the reported spectrophotometric and titrimetric methods because of its higher sensitivity. The method can be applied for the quality control analysis of PEF containing dosage forms without interference.

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