Egyptian Journal of Chemistry

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Indirect Spectrophotometric Estimation of Ciprofloxacin Hydrochloride in Pharmaceuticals using N-Bromosuccinimide and Methylene Blue Dye

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Abstract

The present study aims to develop a swift , simple, accurate and sensitive spectrophotometric method for determination of Ciprofloxacin Hydrochloride (CPFX) in pure and pharmaceutical formulations. The suggested method depend on oxidation of CPFX with known excess amount of N-bromosuccinimide (NBS) in acidic medium and after reaction is insured to be complete , the surplus NBS is estimated by de colorization of methylene blue dye and measuring the absorbance of surplus dye at 665 nm . A linear calibration curve was obtained over the concentration range 0.5 – 4.5 µg.ml^{-1} with correlation coefficient of 0.9993 . The molar absorptivity and sandell's sensitivity index values were determined to be 8.6 ×10^{4} L.mol^{-1}.cm^{-1} and 0.004276 µg.cm^{-2}, respectively .The limit of detection (LOD) and quantification (LOQ) were calculated to be 0.0033 and 0.0111 µg.ml^{-1}, respectively . The proposed method has been successfully applied to the determination of CIPH in available dosage form, the validity proposed method was confirmed by recovery study via standard addition technique .

Keywords: *Ciprofloxacin Hydrochloride ; N-bromosuccinimide ; Determination ;Spectrophotometry ; Methylene blue*

1.Introduction

Ciprofloxacin Hydrochloride (CPFX), chemically known as 1-cyclopropyl – 6 –fluoro – 4 – oxo – 7 – (piperazin – 1 – yl) - 1,4- dihydroquinoline – 3 – carboxylic acid hydrochloride (Fig. 1) , is a pale yellow, slightly hydroscopic, crystalline powder, soluble in water, very slightly soluble in anhydrous ethanol, practically soluble in acetone , in dichloromethan, and in ethyl acetate[1,2] .

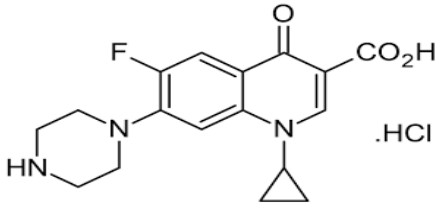

Fig. 1 : chemical structure of ciprofloxacin hydrochloride
M.Wt. = 367.8 g /mol

It was patented in 1983 by Bayer A., Grass, and subsequently approved by the united states in 1987, is a relatively new, second generation fluoroquinoline antibacterial agent with a broad spectrum antimicrobial activity against a variety positive and negative gram bacteria. Ciprofloxacin is mainly used in the treatment of respiratory infections (Klebsiella, Pneumeniae, Pseudomonas aeruginosa, Haemophilus influenzae), urinary tract infections, typhoid fever, for gastrointestinal surgery, streptococcus faecalis, Enterobacter aerogenes, gonorrhoeab (enterotoxigenic strains of escherichia coil), skin structure infections, bone and joint infections, soft tissues and seplicaemia. It is also used in prophy laxis of infections in cancer patients. It acts via inhibition of tow essential bacterial – enzymes : DNA gyrase and topoisomerases IV[3-5].

Due to the medicinal importance of the ciprofloxacin from medical point of view and due to widespread use of this group of drugs, several analytical techniques have been reported in scientific literature for the determination of drug in Pharmaceutical preparations and biological fluids such as titrimetry[6-8],Rayleigh light scattering technique(RLS)[9-10], Voltammetry[11-13], Turbidimetry[14], Capillary electrophoresis[15,16], electrochemical methods[17-19], Spectroflurimetry[20-22], Atomic absorption spectrometry[22,23], High – performance liquid chromatography[24-26],

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Receive Date: 09 June 2021, Revise Date: 01 July 2021, Accept Date: 11 July 2021
DOI: 10.21608/EJCHEM.2021.79851.3930

Chemiluminescence [27], (UV – VS)spectrophotometry[22,28-36],Spectroscopic and Thermal studies[37], Optical sensor[38] and Study of properties of new complexes of ciprofloxacin[39].

However, several of these procedures requires expensive equipment and skilled operation. Literature survy revealed that there were no methods reported for the determination of ciprofloxacin hydrochloride by oxidative spectrophotometric technique with more accuracy and less time. The aim of present paper is to develop a swift, simple, accurate, validate and sensitive spectrophotometric method for the estimation of ciprofloxacin in pure form and it's pharmaceuticals based on the oxidation of ciprofloxacin by using N-bromosuccinimide the colour palace of methylene blue dye by the unreacted N-bromosuccinimide.

EXPERIMENTAL

2.Apparatus

All absorption spectra and absorbance measurements were done by using a double beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm quartz cells. professional Benchtop pH meter BP3001 was used for the pH measurements **.**

2. Chemical reagents

All chemicals used in this study were of analytical reagent grade. Ciprofloxacin hydrochloride was purchased from the State Company for Samarra Drug Industries and Medical Applianes (SDI).

2.1. *CPFX solution*

Stock solution (100 µg. ml–1) was prepared by dissolving 0.01 g of pure CPFX in distilled water, then diluted to 100 ml with distilled water using a volumetric flask. Working standard solution **(10 µg. ml^{-1})** was obtained by suitable dilution of stock solution.

2.2. *N-bromosuccinimide solution, 100 µg. ml^{-1} :*

It was prepared by dissolving 0.01 g of NBS in distilled water and diluted to 100 ml with distilled water. This solution was stable for at least three days.

2.3. Methylene blue

stock solution(2 ×10^{-3} mol.L^{-1}) was prepared by dissolving 0.0639 g of dye powder (BDH) in distilled water and diluting to 100 ml with distilled water. Working standard solution (1.8×10-4mol.L-1) was obtained by suitable dilution of stock solution with distilled water.

2.4. *Hydrochloric acid, (2M)*

It was prepared by diluting 16.6 ml of concentrated hydrochloric acid to 100 ml with distilled water.

3. General procedure

A 0.5 ml of 2 mol.L^{-1} HCl was added to a series of 10 ml volumetric flasks, followed by adding 1.6 ml of 100 µg. ml^{-1} NBS solution. An increasing volume of 10 µg. ml^{-1} of CPFX solution was added to cover the rang 0. 5-4.5 µg.ml^{-1}. The solutions were allowed to stand at room temperature for 10 min, Then 1.5ml of 1.8×10^{-4}mol.L^{-1} MB solution was added, then stand for 5 min and complete to mark with distilled water. The absorbance was measured against reagent blank similarly prepared without drug at 665nm.

4. Procedure for dosage forms

4.1. CPFX tablets

Ten tablets of each (MICROFLOX. INDIA, CIPRONEER. IRAQ) were weighed, crushed into fine powder. A portion of powder equivalent to 0.01 g was weighed and dissolved in distilled water, stirring and mixed well with heating , then filtrated using filter paper, the filtrate was transferred into 100 ml volumetric flask and the volume was completed to the mark with distilled water. This solution was treated as in a general procedure.

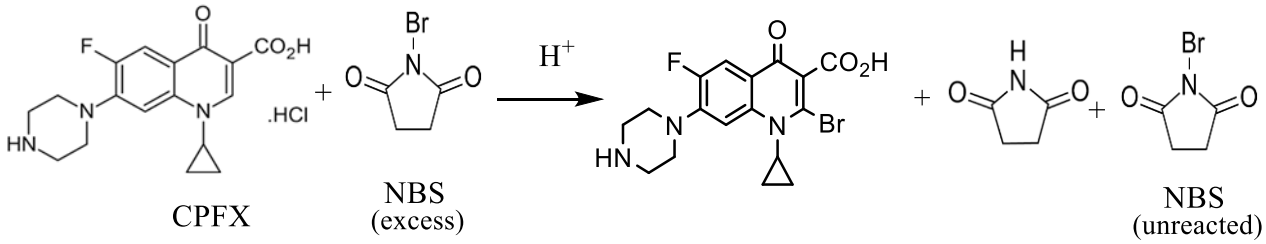
4.2. Eye drops

Contents of three drops of each (Ciproneer. Iraq, CIPROCIN. Jordan) were mixed and 1 ml of mixture was transferred into 100 ml volumetric flask and diluted to the mark with distilled water to obtain 30 µg. ml^{-1}. This solution was treated as in a general procedure.

5. RESULTS AND DISCUSSION

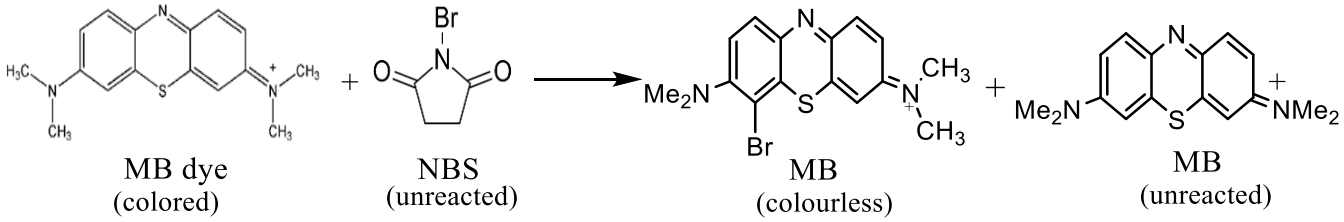
5.1. Principle of method and suggested chemical reaction

Frome follow – up to the literature and research of kinetic and mechanism reactions, we note that NBS is an oxidizing agent and a bromination agent in the acid medium of aliphatic and aromatic organic compounds [40], so suppose a chemical reaction between CPFX and calculated amount of NBS (Scheme1).



Scheme 1

Then the unreacted NBS is react with MB dye and oxidized a fixed amount of MB dye to colourless result, finally measured the residual amount of dye at 665 nm which is proportional to concentration of CPFX (Scheme 2).



Scheme 2

*6. **Optimum reaction conditions***

The following experiments were conducted in 10 ml volumetric flasks with 20 µg of CPFX and measuring MB dye absorption at 665 nm

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6.1. Amount of MB dye

To find the largest amount of MB dye that can be used in the determination of CPFX which that followed the Bear's law, increasing volumes of 1.8×10^{-4} mol.L^{-1} MB dye solution were added to 10 ml volumetric flask contained 1 ml of (2 M) hydrochloric acid. The volume was completed to the mark with distilled water and the absorbance was measured at 665 nm. The standard curve as in (fig.2) shows that 1.5 ml of dye is the best volume that gives highs absorbance within the linear relationship.

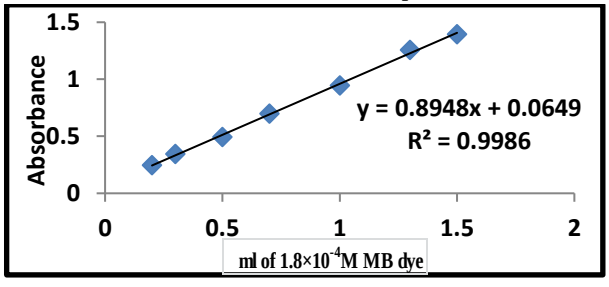

Fig.2: Standard curve for MB dye

6.2. Choose of oxidant agent

This study was done by adding 1 ml of available oxidizing agents that decolorized MB dye (N-bromosuccinimide, N-chlorosuccinimide, Potassium periodate and ChloramineT) with concentration (100 µg. ml^{-1}) of each one into 10 ml volumetric flask which contain 1.5 ml of 1.8×10^{-4} mol.L^{-1} MB dye and 1 ml of 2 M HCl, then volume was completed to the mark with distilled water. The solution was stand for 10 min. and the absorbance was measured at 665 nm. (fig. 3) shows that NBS gives the best results, so it was chosen in the subsequent experiments.

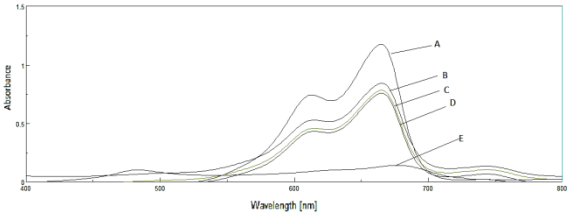

Fig.3:(A) MB dye without oxidant (B) MB dye with N-chlorosuccinamid, (C) MB dye with potassium periodate, (D) MB dye with chloraminT, (E) MB dye with N-bromosuccinamid

6.3. Amount of oxidant agent

NBS volume was studied by changing the reagent volume while the other factors were constant. It found that 1.6 ml of 100 µg. ml^{-1} NBS is sufficient for decolorization of MB dye (fig. 4).:

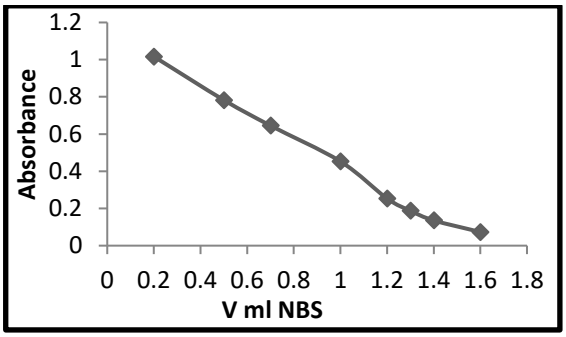

Fig.4: Amount of oxidant agent for de colorization

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6.4. Effect of acidity

Different types of acids were studied, it was found that hydrochloric acid is an ideal medium for the reaction (table 1). In addition 1.5 ml of 2 M HCl was chosen as optimum amount (table 2):

Table 1:Choose the type of acid

Acid solution 1 ml of (2M)	Absorbance	pH
HCl	0.501	1.6
H_2SO_4	0.381	1.8
HNO_3	0.121	1.5
CH_3COOH	0.016	3.2
H_3PO_4	0.201	2.1

Table 2: Choose amount of acid

ml of 2M HCl	Absorbance / µg of CPFX			
	10	20	30	40
0.2	0.151	0.424	0.607	0.893
0.4	0.197	0.463	0.680	0.912
0.5	0.255	0.515	0.732	0.981
0.6	0.201	0.481	0.652	0.871
0.8	0.179	0.406	0.598	0.809

6.5. Effect of oxidation time

Time is important for oxidation reaction. It has been determined by adding reaction contents before adding MB dye. After shaking the flasks and waiting for suitable time, MB dye was added. Again shaking the flasks for several minutes to complete oxidation reaction. (table 3) shows that 10 min. is the better time for oxidation of CPFX. Also 5 min. was suitable time for oxidation of MB dye.

Table 3: Effect of oxidation time

Standing time before addition MB , min	Absorbance / Standing time after addition MB and before dilution, min							
	5	10	15	20	30	40	50	60
After addition	0.477	0.479	0.481	0.479	0.478	0.478	0.472	0.473
5	0.493	0. 494	0. 495	0.501	0.499	0.504	0.501	0.506
10	0.519	0.518	0.517	0.510	0.509	0.511	0.508	0.507
15	0.515	0.516	0.515	0.517	0.513	0.511	0.507	0.507
20	0.499	0.494	0.493	0.484	0.473	0.471	0.473	0.469

6.6- Effect of temperature and stability

The stability of reaction was studied in different temperatures and (table 4) shows that the absorbance is stable for one hour at room temp.(25C° ± 2).

Table 4: Effect of temperature and stability

Temp. C°	Absorbance of 20 µg of CPFX / min standing								
	5	10	15	20	25	30	40	50	60
0	0.375	0.376	0. 375	0.374	0.376	0.375	0.375	0.376	0.376
10	0.455	0.455	0.456	0.457	0.453	0.453	0.455	0.454	0.452
RT=25	0.518	0.518	0.519	0.518	0.517	0.518	0.518	0.517	0.518
40	0.440	0.441	0. 440	0.442	0.443	0.440	0.442	0.442	0.441

6.7. Effect of surfactants

Different types of surfactants (non-ionic, anionic and cationic) were studied. It was found that these surfactants decreased the absorbance as in the (table 5), therefore, omitted in this study.

Table 5: Effect of surfactants

Surfactants	Absorbance / ml of surfactants				
	0.0	0.5	1	2	3
Triton X-100 2 %	0.517	0.498	0.500	0.497	0.498
SDS 1×10^{-3} M	0.517	0.488	0.489	0.492	0.488
CTAB 1×10^{-3} M	0.517	0.501	0.500	0.499	0.496
CPC 1×10^{-3} M	0.517	0.490	0.491	0.486	0.480

6.8. Final absorption spectrum and calibration curve

When CPFX is reacted according to the general procedure, the absorption spectra is shown in (fig. 5) shows maximum absorption at 665 nm. A linear calibration curve (fig. 6) is obtained over the range (0.5 – 4.5)µg. ml^{-1} with molar absorptivity 8.6×10^4 $L.mol^{-1}.cm^{-1}$ and sandell's sensitivity 0.004276 $\mu g.cm^{-2}$. The limit of detection(LOD) and limit of quantification (LOQ) were calculated by taking replicates for blank and the absorption was measured versus distilled water at 665 nm.

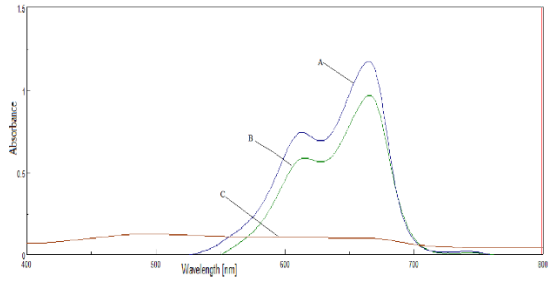

Fig.5: Absorbtion spectra of (A) MB dye only , (B) MB dye with 40 µg of CPFX/ 10 ml vs. blank , (C) blank vs. distilled water

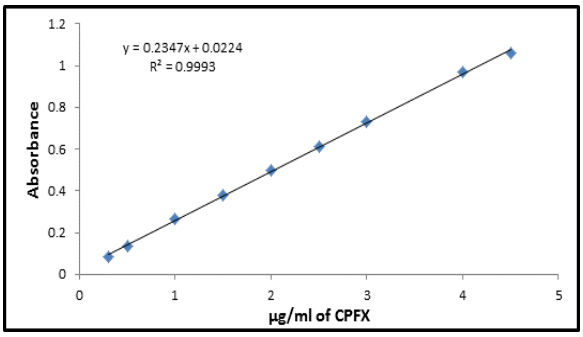

Fig. 6 : Calibration curve of ***CPFX*** Estimation

6.9. Accuracy and precision

To calculate the accuracy and precision of the calibration curve, CPFX is determined at two concentrations. Results illustrated in (table. 6) shows that these are reliable.

Table 6: Accuracy and precision

Amount of CPFX µg/10 ml	Recovery %	Rrelative error %*	Relative standard deviation %*
10	98.43	1.56	±0.745
20	100.97	0.97	±0.608

*Average of five determinations

6.10. Effect of interferences

The effect of the presence of some drug additives that are usually present in pharmaceutical preparations has been studied under optimal conditions by adding different amounts of these additives to 20 µg CPFX /10 ml. The results obtained in (table 7) shows on significant interference from these material, indicating the efficiency, selectivity of the method for its applications of the pharmaceutical preparations.

7. Application of the method

The suggested method was successfully applied to determine the drug in their commercial preparations (tablets & eye drops). The results in (table 8) indicated that the method is accurate and reproducible.

Table 7: Effect of interferences

Interference	Recovery % of 20 µg CPFX / µg of interferences			
	20	40	100	200
Starch	99.39	99.22	99.02	98.44
Glucose	99.61	99.41	97.27	97.46
Arabic gum	99.41	100.19	100.03	100.01
Succrose	99.62	99.39	99.22	99.31
Lactose	99.37	100.07	100.11	99.62

Table 8: Application of the method

Pharmaceutical preparations	Amount of CPFX µg/10 ml	Recovery %	Rrelative error %*	Relative standard deviation %*
MICROFLOX, 500 mg/Tab. India	10	98.43	1.56	±0.48
	20	100.97	0.97	±0.192
CIPRONEER, 500 mg/Tab. Iraq	10	102.27	2.27	±1.34
	20	101.24	1.24	±1.171
Ciproneer CPFX 0.3 % Iraq	10	97. 8	2.19	±1.189
	20	100.27	0.27	±0.401
CIPROCIN, CPFX 0.3 % Jordan	10	101.88	1.88	±0.74
	20	100.77	0.77	±0.304

*Average of five determinations

8.2. Evaluation of the suggested method

The standard addition procedure was applied to confirm the validity of the method by adding constant volume of the solution containing a fixed amount of pharmaceutical preparation to series 10 ml volumetric flasks. Then an increasing volumes of stock solution were added. Finally, each flask is made up to the mark with distilled water and mixed well. The abs. of solutions was measured at 665 nm. (fig. 7) and the results in (table 9) which exhibit a good agreement between standard addition method and suggest method.

9. Comparison of the method

The accuracy and sensitivity of the proposed method by measuring some analytical variables for the determination of CPFX was compared with some spectrophotometric methods proven in the literature as shown in(table 10).

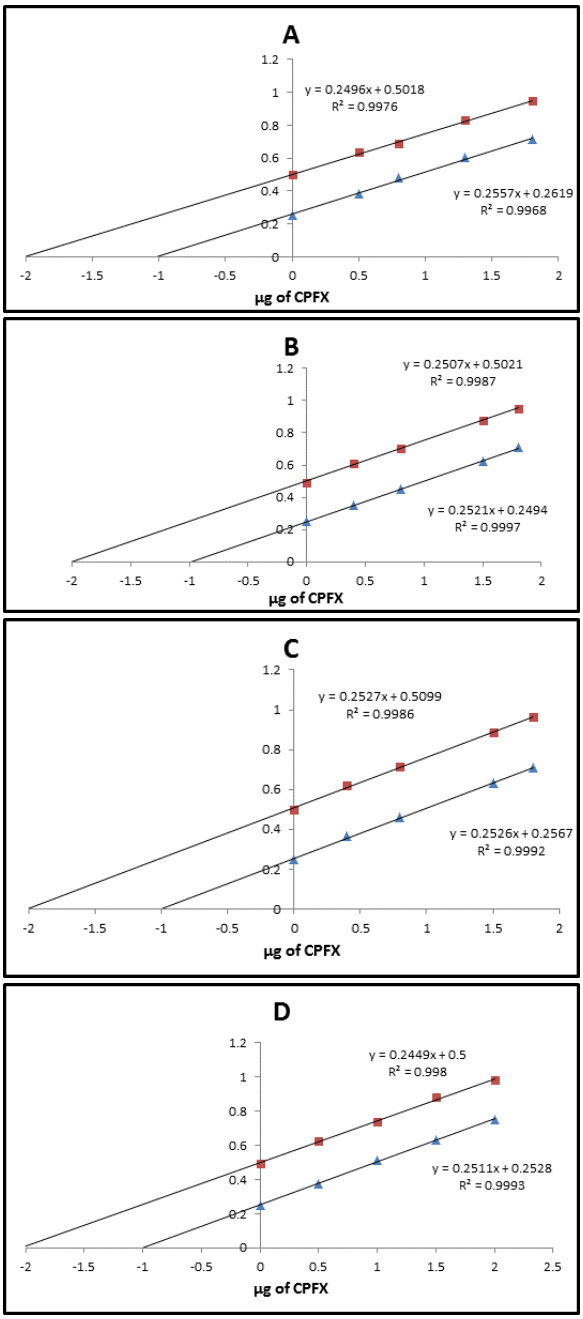

Fig.7: Standared addition curves for estimation of CPFX preparations (A) MICROFLOX tablets - India (B) CIPRONEER Tablets-Iraq (C) Ciproneer eye drops - Iraq (D) CIPROCIN eye drops – Jordan

Table 9: Determination of CPFX in pharmaceuticals by standared addition method

Pharmaceutical preparations	Amount of CPFX taken µg/ ml	Amount of CPFX measured µg/ ml	Recovery %
MICROFLOX, 500mg/Tab. India	1	1.024	102.4
	2	2.010	100.5
CIPRONEER, 500mg/Tab. Iraq	1	0.989	98.9
	2	2.002	100.1
Ciproneer, CPFX 0.3 % Iraq	1	1.016	101.6
	2	2.017	100.9
CIPROCIN, CPFX 0.3 % Jordan	1	1.006	100.6
	2	2.042	102.0

Table 10: Comparison of the method

Parameter	Present method	Literature method [33]	Literature method [35]
Type of reaction	Oxidation - reduction	Oxidation - reduction	Oxidation - reduction
Oxidant agent	N-bromosuccinimide	Cerium (IV) ammonium sulfate	Potassium permanganate
reagent	Methylene blue dye	Amaranth dye	----------
λ_{max} (nm)	665	523	525
Medium of reaction	Acidic	Acidic	Acidic
Beer's law range(ppm)	0.5 – 4.5	1.2 – 8.4	4 – 18
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	8.6×10^4	5.11×10^4	1.47×10^4
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0042	0.0380	0.0225
Recovery (%)	98.43 -100.97	98.33 -100.66	97.46 -100.24
Color of product	Blue	Wine – red	----------
Application of the method	pharmaceutical preparations	pharmaceutical preparations	pharmaceutical preparations

10.CONCLUSION

In this paper, an indirect spectrophotometric method using N-bromosuccinimide and methylene blue dye was used for the first time for the determination of ciprofloxacin hydrochloride . The method was swift, validate, highly selective and sensitive, and it was characterized by simplicity because it did not need to be organized the temperature and extraction steps. In addition, the method was efficient for routine analysis of this class of antibiotics in pharmaceuticals dosage over a wide concentration range without interferences from common excipients. Therfore, the method has been successfully applied to pharmaceutical preparations with good accuracy and precision.

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