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Extractive spectrophotometric methods for determination of ciprofloxacin in pharmaceutical formulations using sulfonephthalein acid dyes

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Abstract

Three simple, rapid, sensitive and accurate extractive-spectrophotometric method for the determination of ciprofloxacin in pharmaceutical preparation has been developed. These methods are based on the formation of yellow ion-pair complexes between the examined drug and three sulfonephthalein acid dyes, namely; bromophenol blue (BPB), bromocresol green (BCG), and bromothymol blue (BTB) in acidic medium. The formed complexes were extracted with chloroform and measured at 420, the colored chromogen was stable for twenty four hours. The effect of optimum conditions via pH, dye concentration, time and solvent are studied. Beer's law is obeyed in the concentration ranges 0.50-25.0 µg/mL with molar absorptivity of 1.46×10^4, 1.83×10^4 and 2.07×10^4 L. mol^{-1}. cm^{-1} and limit of detection (LOD) of 0.105, 0.101, 0.084 for BPB, BCG and BTB methods, respectively. No interference was observed from common excipients present in pharmaceutical formulations. The proposed method has been applied successfully to determine ciprofloxacin in pharmaceutical preparation (tablets, infusion and eye drops).

Keywords. Ciprofloxacin, extraction-spectrophotometry, ion pair complex; sulfonephthalein dyes.

1. INTRODUCTION

Ciprofloxacin (CPF), 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid (Fig.1), is a second generation fluoroquinolone antibacterial agent with a broad spectrum of activity against a variety of gram positive and gram negative bacteria. It is widely used in the treatment of acute sinusitis, lower respiratory tract infection, urinary tract infection, chronic bacterial prostatitis and non-complicated intra abdominal infections caused by *E. coli, P. aeruginosa, Proteus mirabilis* when used in combination with metronidazole[1].

A number of analytical methods have been reported for the determination of ciprofloxacin in pharmaceutical dosage forms and biological fluids including spectrofluorometric, micellar electrokinetic chromatography, differential pulse voltammetry, flow injection analysis, high-performance liquid chromatography, liquid chromatography tandem mass spectrometry and spectrophotometric [2-4]. Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories. Spectrophotometric methods reported for the determination of ciprofloxacin include oxidative coupling with 3-methyl-2-benzothiazolinonehydrazone hydrochloride (MBTH) and cerium (IV) ammonium sulfate, Fe(III)- MBTH, Fe(III)-1,10-phenanthroline, Fe(III)-Bipyridil [5-6], charge-transfer complexation with p-acceptors such as 2,3-dichloro-5,6-dicyano-q-benzoquinone, 7,7,8,8-tetracyanoquinodimethane (TCNQ), p-chloranil, p-nitrophenol and tetracyanoethylene [7], ion-pair complex formation with acid–dye reagents such as cobalt (II) tetrathiocyanate, Bi (III) tetraiodide, sudan III, methyl orange, supracene violet 3B, tropeolin 00, bromophenol blue, bromothymol blue or bromocresol purple [8-11].

However, the disadvantages of using these methods are that the reaction is often narrow linearity range, requiring heating, long time for the reaction to complete, low stability of the colored product formed.

Bromophenol blue (BPB), bromocresol green (BCG) and bromothymol blue (BTB) are known to yield an ion-pair complex, which are applied in the determination of many pharmaceutical compounds by extractive spectrophotometric[12-13]. The methods based on ion pair complexes extractable into a suitable organic solvent have been shown to be simple, sensitive, accurate and economical.

In this paper we report three simple, rapid and sensitive extractive spectrophotometric methods for the determination of ciprofloxacin in pharmaceutical formulations. The methods are based on ion-pair complexation between ciprofloxacin with anionic dye namely bromophenol blue (BPB), bromocresol green (BCG) and bromothymol blue (BTB) subsequent extraction into chloroform and measure the absorbance of color complex. The proposed methods were applied to the determination of ciprofloxacin in pharmaceutical preparation.

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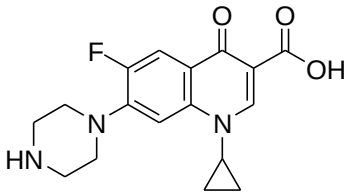

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Figure 1: Chemical structure of ciprofloxacin

2. EXPERIMENTAL

2.1. Chemicals and equipment

All chemicals used were of analytical grade and double distilled water was used for dilution of reagents and samples. Ciprofloxacin hydrochloride (Sigma-Aldrich, Germany, certified to be 99.0%), bromophenol blue (BPB), bromocresol green (BCG) and bromothymol blue (BTB) (Maya - R, China, certified to be 99%) were used. The most common solvents are chloroform, dichloromethane, carbon tetrachloride, dichloroethane, benzene, toluene, n-hexane and other chemicals used were of analytical reagent grade.

The following dosage forms containing ciprofloxacin were purchased from local pharmacy market and employed in the study: 1 – Hasancip and Kacipro tablets equivalent to 500 mg ciprofloxacin (Hasan-Dermapharm and Dong Nam manufacturing – Trading pharmaceutical Co., Ltd, Viet Nam), 2 – Ciprofloxacin infusion equivalent to 200 mg ciprofloxacin /100 mL solution for infusion (Hebei Tiancheng Pharmaceutical Co., Ltd and Shandong Hualu Pharmaceutical Co., Ltd, China) and 3 – Ciprofloxacin 0,3% eye drops equivalent to 30 mg ciprofloxacin/10 mL solution (Thanh Hoa pharmaceutical and mediacal supplies joint stock company, Viet Nam).

A Biochrom Model SP-60 double beam, UV-VIS spectrophotometer (Biochrom Ltd., UK) with 1.0 cm matched quartz cells was used for absorbance measurements.

2.2. Standard solutions

A stock solution of ciprofloxacin (1mg/mL) in double distilled water. The working standard solution of ciprofloxacin containing 100 µg/mL was prepared by dilution.

The dyestuffs were used as 0.025 % solutions in doubly distilled water.

2.3. Pharmaceutical preparations of ciprofloxacin

Tablets: Weigh and mix the contents of twenty tablets (each one contains 500 mg ciprofloxacin), an accurately weighed amount of powder equivalent to 0.1g of CPF transferred in to a 100 mL beaker. Using a magnetic stirrer, the powder was completely disintegrated in doubly distilled water, filtered through a Whatman filter paper No 40 and diluted up to 100 mL with doubly distilled water in a volumetric flask. The working solution of the drug containing 100 µg/mL was prepared by dilution and the below procedure was followed.

Infusion solution (2 mg/mL) and eye drops (30 mg, 10 mL each): a suitable volume was diluted to 100 µg/mL with double distilled water and the below procedure was followed.

2.4. Procedure and calibration graph

Into a series of 125 mL separating funnel, volumes of CPF working standard solution equivalent to 0.5-25 µg/mL were transferred. To each funnel, add 4.0 mL of 0.025% BPB, BCG, BTB, respectively and mixed well. Then 10 mL of chloroform was added to each of the separating funnel. The contents were shaken for 2 min and allowed to separate the two layers. The absorbance of the organic phase at 420 nm was measured in each case against a reagent blank similarly prepared and and a calibration graph was constructed. The colored chromogen was stable for twenty four hours.

2.5. Statistical analysis

Method was validated according to ICH Guidelines[14], in terms of linearity and range, accuracy and precision, limit of detection (LOD), limit of quantitation (LOQ).

Calculation and processing of data were done using the programs Origin Pro 8.0 (USA).

3. RESULTS AND DISCUSSION

3.1. Principles of the method

Ciprofloxacin contains a secondary amino group, which is protonated in acid medium, while sulphonic acid group is present in BPB, BCG and BTB that is the only group undergoing dissociation in the pH range 1-5. The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, the protonated ciprofloxacin forms ion-pairs with anionic dyes, which are quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in figure 2.

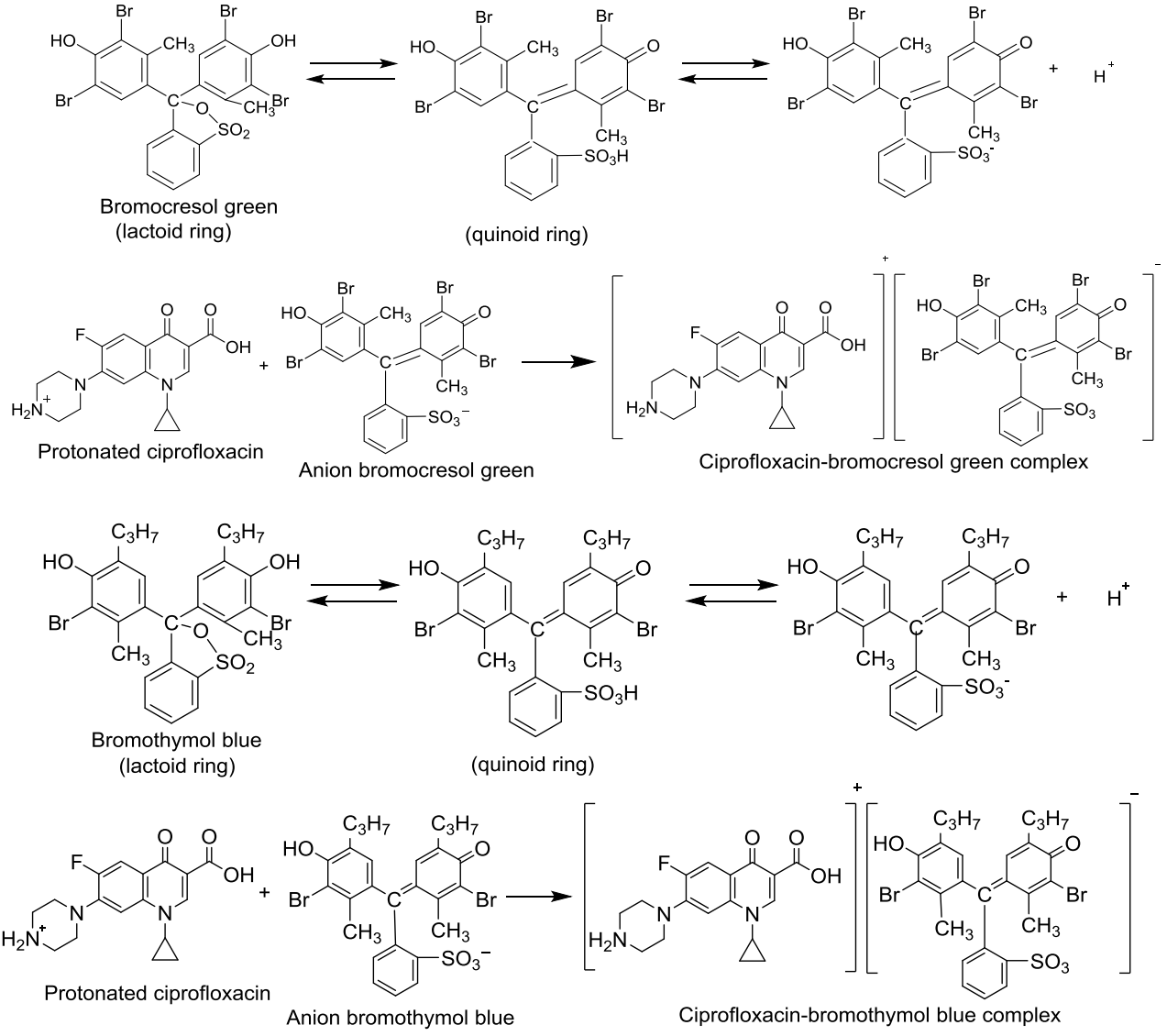

Figure 2: The possible reaction mechanism for the reaction between ciprofloxacin and bromocresol green, bromothymol blue

3.2. Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. The following parameters were optimized such as dye concentration, type of extracting solvent, effect of pH and effect of shaking time.

3.1.2. Effect of pH

The influence of pH on the ion-pair formations of ciprofloxacin with various dyes has been studied using HCl 1 M and NaOH 1 M. It was noticed that the highest absorbance value were observed at pH

3.3, 3.4 and 3.5 for BPB, BTB and BCG method, respectively (Fig. 3). Thus, all the absorbance measurements were made at pH 3.3, 3.4 and 3.5 with BPB, BTB and BCG, respectively.

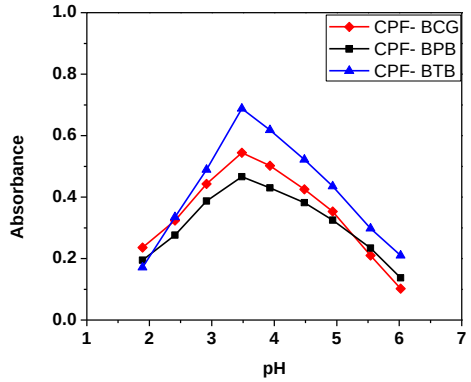

Figure 3: Effect of pH on the absorbance of 10 μg.mL^{-1} CPF acid-dye

3.2.2. Effect of dyestuff concentration

The effect of dyestuff concentrations was also studied by adding different volumes of 0.025 % dyestuff (0.5-7.0 mL) to a constant amount of CPF(10 μg.mL^{-1}). The results showed that the maximum color intensity of the complex was achieved with 4.0 mL of 0.025 % of each dye. Thus, 4 mL of each dyestuff was used for ion-pair formation throughout the experiment (Fig. 4).

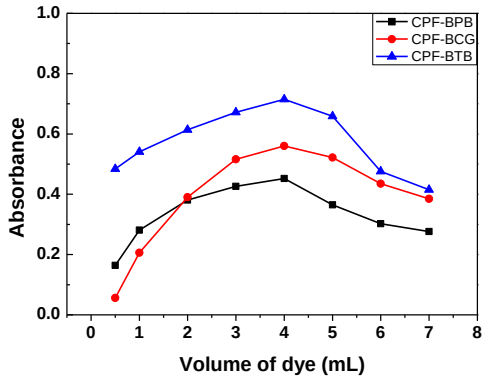

Figure 4: Effect of the volume of 0.025 % dyes with 10 μg.mL^{-1} CPF

3.2.3. Effect of extracting solvent

A number of organic solvents such as chloroform, carbon tetrachloride, dichloromethane, benzene and toluene were examined for extraction of the ion-pair complex in order to provide an applicable extraction procedure. The most convenient solvent found to produce the highest absorbance, extraction power and stability of color of the formed ion-associates was chloroform for BCG, BPB and BTB (table 1).

Table 1: The effect solvent that required for ion-pair complex formation (λ_{max} = 420 nm)

Organic solvent	Absorbance (10 μg/mL of CPF)		
	BPB	BCG	BTB
Chloroform	0.452	0.565	0.695
Dichloromethane	0.436	0.553	0.663
Dichloroethane	0.182	0.325	0.140
Carbon tetrachloride	0.033	0.046	0.047
Benzene	0.028	0.044	0.175
Toluene	0.133	0.008	0.074

3.2.4. Effect of shaking time

The effect of shaking time on the formation and stability of the ion-pair complex was studied by measuring the absorbance of the extracted ion-associates at increasing time intervals (0-4.0 min), the results showed that the ion-pair complex were formed almost instantaneously in all cases at room temperature with 2.0 min shaking time (Fig. 5). The absorbances of the complexes were found to be stable for more than 24 h.

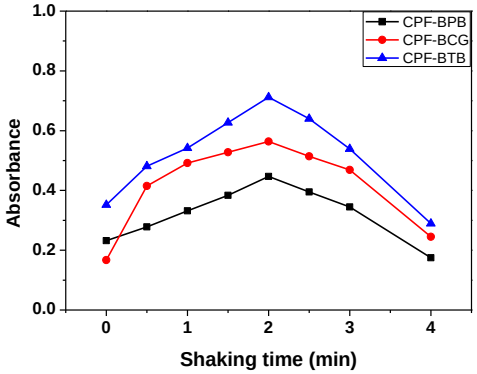

Figure 5: Effect of shaking time on the ion pair complexes

3.2.5. Composition of ion-pair complexes

Job's method of continuous variation of equimolar solutions was employed: a 3.0×10^{-4}M standard solution of ciprofloxacin and 3.0×10^{-4}M solution of BPB, BCG and BTB, respectively, were used. A series solutions was prepared in which the total volume of drug and reagent was kept at 10 mL for BPB, BCG and BTB, respectively. The absorbance was measured at 420 nm for each dye. The molar ratio of the reagents (drug:drug+dye) in the ion-pair complexes was determined by the method

continuous variations (Job's method) (Fig. 6).The results indicate that 1:1 (drug:dye) ion-pairs are formed through the electrostatic attraction between positive protonated CPF^+ and negative BPB^-, BCG^- and BTB^-. The extraction equilibrium can be represented as follows:

$$CPF^+_{(aq)} + D^-_{(aq)} \rightleftharpoons CPF^+ D^-_{(aq)} \rightleftharpoons CPF^+ D^-_{(org)}$$

where CPF^+ and D^- represent the protonated ciprofloxacin and the anion of the dye, respectively, and the subcript (aq) and (org) refer to the aqueous and organic phases, respectively.

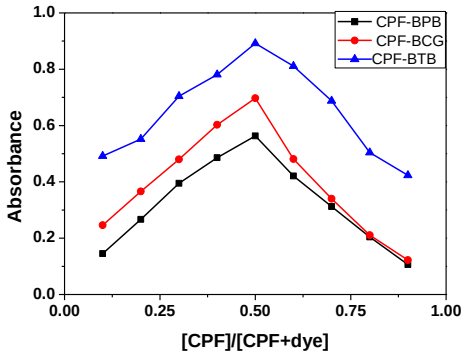

Figure 6: Job's method of continuous variation graph for the reaction of ciprofloxacin with acid-dyes BPB BCG and BTB, [drug]=[dye] = 3×10^{-4} M

3.2.6. Spectral characteristics

The absorption spectra of the ion-pair complexes extracted into chloroform are shown in Fig. 7. The ion-pair complexes with BTB, BPB and BCG absorbed maximally at 420 nm. The reagent blank under similar conditions showed no absorption.

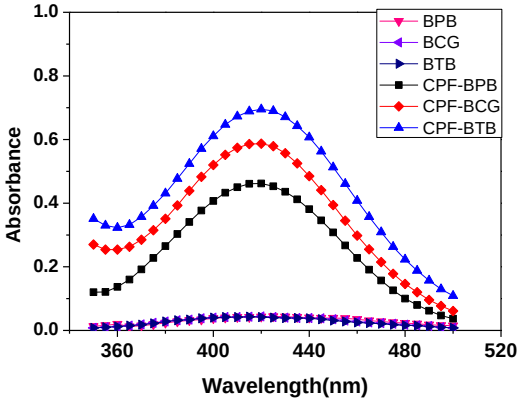

Figure 7: Absorption spectra of ciprofloxacin (10µg.mL^{-1})-dye complex extracted into 10 mL chloroform

3.3. Validation of the proposed method

The proposed methods are successfully validated according to International Conference on Harmonization (ICH) guidelines [14]. The limit of detection (LOD) and quantification (LOQ) of the method are given by $3{,}3.\frac{SD}{b}$ and $10.\frac{SD}{b}$ respectively, relative standard deviation (RSD(%)= $\frac{SD}{\bar{X}}.100$; where SD is the standard deviation of blank absorbance values, b is the slope of the calibration curve equation, $\bar{X}$" is the average value of the measurement. Blank samples were prepared as described in section 2.4 but without CPF.

Under the described experimental conditions, calibration curves for proposed methods were constructed (Fig. 8). The linear regression equations, standard deviation, slopes and intercepts, correlation coefficients, relative standard deviation of response factors, and linearity ranges were given in (table 2) for each proposed spectrophotometric method. The molar absorptivities, Sandell's sensitivity of each methods was calculated and these values showed that the molar absorptivity of BTB > BCG > BPB ion-pair complexes.

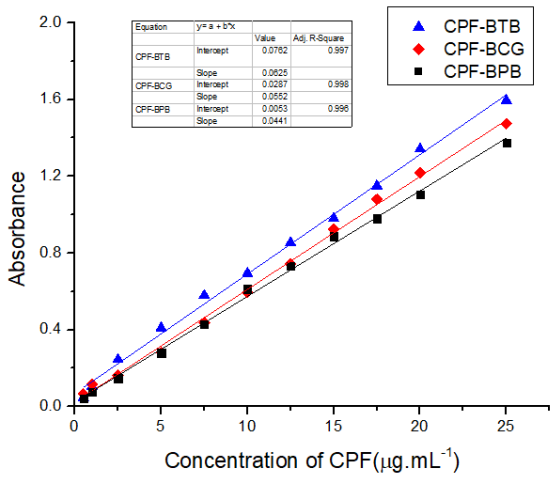

Figure 8: Standard curves of CPF ion pairs with BTB, BCG and BPB at λ_{max} 420 nm

The accuracy and precision of the methods were determined by preparing solutions of three different concentrations of ciprofloxacin and analyzing them in six replicates. Samples for analysis were prepared as described in section 2.4. The precision of the proposed methods was evaluated as percentage relative standard deviation (RSD%) and accuracy as percentage relative error (RE%). The percentage relative error calculated using the following equation: RE(%) =[(founded–added)/ added]x 100

The accuracy and precision results are shown in table 3.

The low values of the relative standard deviation percentage and relative error percentage specify the high precision and the good accuracy of the method.

Table 2: Analytical characteristics of the proposed methods (n = 6)

Parameters	Proposed methods		
	BPB	BCG	BTB
Colour	Yellow	Yellow	Yellow
Wavelengths λ_{max} (nm)	420	420	420
pH	3.3	3.5	3.4
Stability (h)	24	24	24
Shaking time (min)	2	2	2
Stoichiometric ratio	1:1	1:1	1:1
Beer's law range ($\mu g.mL^{-1}$)	0.5-25	0.5-25	0.5-25
Limit of detection ($\mu g.mL^{-1}$)	0.105	0.101	0.084
Limit of quantitation ($\mu g.mL^{-1}$)	0.315	0.303	0.252
Molar absorbitivity ($L.mol^{-1}.cm^{-1}$)	$1.46x10^4$	$1.83x10^4$	$2.07x10^4$
Sandell's sensitivity ($\mu g/cm^2$)	0.0686	0.0562	0.0462
Regression equation (Y = bx +a)			
Slope (b)	0.0441	0.0552	0.0625
Intercept (a)	0.0053	0.0287	0.0762
Correlation coefficient (R^2)	0.996	0.998	0.997

Table 3: Evaluation of accuracy and precision of the proposed methods (n = 6)

Method	Amount taken ($\mu g.mL^{-1}$)	Amount found ($\mu g.mL^{-1}$)	Recovery (%)	RSD (%)	RE (%)
BCG	5.00	5.08	101.6	1.16	1.60
	10.00	9.97	99.70	0.48	-0.30
	15.00	14.84	98.93	0.75	-1.07
BPB	5.00	5.06	101.2	0.97	1.20
	10.00	10.08	100.8	0.54	0.80
	15.00	14.94	99.60	0.86	-0.4
BTB	5.00	4.94	98.80	1.02	-1.2
	10.00	9.96	99.60	0.57	-0.4
	15.00	15.18	101.2	0.94	1.2

3.4. Effects of interference

The extent of interference by various excipients (magnesium stearate, glucose, lactose, starch and sodium chloride) which often accompany the pharmaceutical preparations was studied in a total volume of 10 mL chloroform. The interference was determined by measuring the absorbance of a solution containing 10 µg/mL of ciprofloxacin. This study was carried out by following the proposed procedures for a 10 mL sample system, by adding a known amount of foreign species to a ciprofloxacin solution of 10 µg/mL. The tolerance limits of interfering species were established at those concentrations that do not cause more than ±2.0% error. The tolerance limits of excipients are listed in table 4. The results indicated that there is no interference from the degradation, indicating a high selectivity for determining the studied ciprofloxacin in its dosage forms.

Table 4: Effect of foreign species on the determination of 10 $\mu g.mL^{-1}$ ciprofloxacin

Excipients	Tolerance limit ($\mu g. mL^{-1}$)
Magnesium stearate	500
Glucose	250
Lactose	500
Sodium chloride	500
Starch	250

3.5. Comparison with other spectrophotometric methods

The proposed method compares favorably with other reported methods. As shown in table 5 the proposed method is more high sensitivity than other methods,

needs no heating, the product is stable for a longer time and and are free from interference with common excipients.

Table 5: Comparison of VIS spectrophotometric methods for ciprofloxacin determination

No.	Reagent	λ_{max} (nm)	Range of determination ($\mu g.\ mL^{-1}$)	Molar absorbitivity ($L.mol^{-1}.cm^{-1}$)	Remarks	Ref.
1	Ce(IV)- MBTH	630	10-50	-	Involves contact time	[5]
3	Fe(III)-1,10-phenanthroline	510	0.04-7.2	3.4×10^4	Involves contact time and heating	[6]
4	Fe(III)-Bipyridil	522	0.05-9	2.95×10^4	Involves contact time and heating	
5	CL	520	16-96	-	Involves contact time and heating	[7]
6	TCNE	335	0.25-15	-	Involves contact time and heating	
7	Co(II) tetrathiocyanate	623	20-240	8.38×10^2	Less sensitive	[8]
8	Sudan III	566	0.4-10.4	2.38×10^4	Involves heating /cooling samples	[9]
9	Eosin Y	547	2-8	3.56×10^4	Less stable colour	[10]
10	Merbromin	545	2-15	1.23×10^4		
11	BPB	420	0.5-25.0	1.46×10^4	Short reaction time, high sensitive and high colour stability	This work
12	BCG	420	0.5-25.0	1.83×10^4		
13	BTB	420	0.5-25.0	2.07×10^4		

MBTH: 3-methyl-2-benzothioazolin-2-one-hydrazone; CL: p-chloranil; TCNE: Tetracyanoethylene; BPB: Bromophenol blue, BCG: Bromocresol green and BTB: Bromothymol blue

3.6. Application of the proposed methods

The proposed method was successfully applied to determine ciprofloxacin in different pharmaceutical preparations (tablets, capsules and eye drops). The results given in table 6 of the analysis showed that the data are consistent with the label claim of the formulations. The relative standard deviation values are below 2 % indicating the precision of the method. The validations of the proposed methods were further confirmed by recovery studies. The % recovery vary from 97.41 to 101.20 indicating high accuracy of methods.

Table 6: Results of ciprofloxacin determination in pharmaceutical preparations

Pharmaceutical preparation	Labeled amount (mg/form)	Recovery (%)			RSD (%)		
		BCG	BPB	BTB	BCG	BPB	BTB
Hasancip tablet	500 mg /tablet	99.75	99.36	98.89	0.59	0.28	0.16
Kacipro tablet	500 mg /tablet	98.36	98.57	101.20	0.74	0.45	0.24
Shandong infusion	200 mg/100 mL	102.53	100.54	97.41	0.43	0.32	0.26
Hebei infusion	200 mg/100 mL	98.05	101.08	97.69	0.87	0.65	0.48
Eye drops	30mg/tube	100.24	99.02	98.53	0.78	0.51	0.39

4. CONCLUSION

This article reports the use of BCG, BPB and BTB as an anionic dyes for the extractive spectrophotometric determination of ciprofloxacin. The performance order of the proposed methods is BTB > BCG > BPB. No interference from common excipients was encountered. The proposed methods are found to be simple, sensitive, selective, accurate, precise, economical and can be used in the determination of ciprofloxacin in different pharmaceutical preparations (tablets, infucsions and eye drops).

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