DETERMINATION OF FLUOROQUINOLONES IN PHARMACEUTICAL FORMULATIONS BY EXTRACTIVE SPECTROPHOTOMETRIC METHODS USING ION-PAIR COMPLEX FORMATION WITH BROMOTHYMOL BLUE

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Abstract

In this paper, we reported a new, simple, accurate, and precise spectrophotometric method for the determination of fluoroquinolones (FQs) including ciprofloxacin (CFX), levofloxacin (LFX) and ofloxacin (OFX) in pharmaceutical formulations. The proposed method is based on the ion-pair formation complexes between FQs and an anionic dye, bromothymol blue (BTB) in acidic medium. The yellow colored complexes which were extracted into chloroform, were measured at the wavelengths of 420, 415, and 418 nm for CFX, LFX and OFX, respectively. Some effective conditions such as pH, dye concentration, shaking time, and organic solvents were also systematically studied. Very good limit of detection (LOD) of 0.084 µg/mL, 0.101 µg/mL, and 0.105 µg/mL were found for CFX, LFX, and OFX, respectively. The stoichiometry of the complexes formed between FQs and BTB determined by Job's method of continuous variation was 1:1. No interference was observed from common excipients occurred in pharmaceutical formulations. The proposed method has been successfully applied to determine the FQs in some pharmaceutical products.

Keywords: *Fluoroquinolones, Spectrophotometric method, Ion-pair formation, Bromothymol blue*

1. INTRODUCTION

Fluoroquinolones (FQs) are the important antibiotics used for the treatment of gram-negative bacterial infections in both human and veterinary medicine. They are derivatives of 4-quinolone, which have unsubstituted or substituted piperazine ring attached at the 7-position to the central ring system of quinoline as well as fluorine atom at the 6-position. The FQs are useful to treat of a variety of infections, regarding to soft tissue infections, respiratory infections, urinary tract infections, bone-joint infections, typhoid fever, prostatitis, sexually transmitted diseases, acute bronchitis, community acquired pneumonia, and sinusitis [1-3].

Ciprofloxacin (CFX), which is one of the second generated group of synthetic FQs, can exhibit greater intrinsic antibacterial activity and make a broader antibacterial spectrum. Ofloxacin (OFX), is a chiral compound that is widely used to treat above infections. Levofloxacin (LFX), is the pure (–)-(S)-enantiomer of the racemic drug substance ofloxacin. Figures 1a, 1b and 1c show the chemical structures of CFX, LFX and OFX, respectively.

Several techniques like voltammetry [4], flow injection electrogenerated chemiluminescence [5], spectrofluorometry [6-7], spectrophotometry [8-9], high performance liquid chromatography [10-11], and liquid chromatography tandem mass spectrometry [12-13] have been used for the determination of fluoroquinolones in pharmaceutical and biological products. Among them, spectrophotometric method has several advantages such as low interference level, good selectivity, simple, fast and low cost. Spectrophotometric was successfully used for pharmaceutical analysis, involving quality control of commercialized product and pharmacodynamic studies. Spectrophotometric methods for the determination of fluoroquinolones could be classified according to the different reactions: (i) Charge-transfer complexation based on the reaction of FQs as electron donors with p-acceptors such as 2,3-dichloro-5,6-dicyano-q-benzoquinone, 7,7,8,8-tetracyanoquinodimethane, q-chloranil, q-nitrophenol and tetracyanoethylene [7, 14-16]; (ii) oxidative coupling reaction using oxidative coupling with 3-methyl-2-benzothiazolinonehydrazone hydrochloride and cerium (IV) ammonium sulfate, Fe(III)- MBTH, tris(o-phenanthroline) iron(II) and tris (bipyridyl) iron(II) [17-18]; (iii) ion-pair complex formation with acid–dye reagents such as sudan III, methyl orange, supracene violet 3B, tropeolin 00, bromophenol blue, bromothymol blue, bromocresol green and bromocresol purple [8, 14, 19-20]. These methods were related with some major drawbacks such as having narrow linearity range, requiring heating and close pH control, long time for the reaction to complete, low stability of the colored product formed.

Bromothymol blue (BTB) (Fig. 1d), is an anionic dye and that can be protonated or deprotonated to form yellow or blue, respectively. The BTB was used to make ion-pair complex, that was applied to determine many pharmaceutical compounds by extractive spectrophotometric [21-25]. However, the ion-pair between BTB and FQs have not been studied. The method based on ion-pair complexes between analytes and BTB into a suitable organic solvent seems to be simple, fast and cheap.

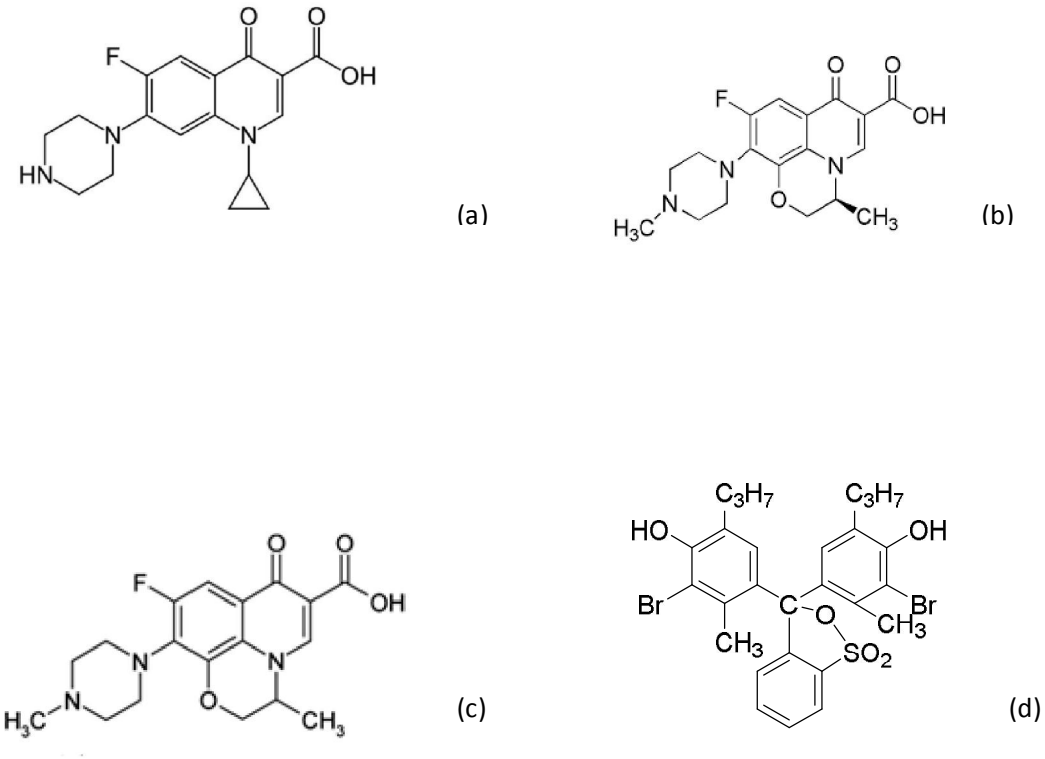


Figure 1: Chemical structures of ciprofloxacin (a), levofloxacin (b) and ofloxacin (OFX) and bromothymol blue (d)

In this paper, for the first time, we investigated extractive spectrophotometric methods based on the formation of ion-pair complexes between ciprofloxacin, levofloxacin and ofloxacin with BTB subsequent extraction into chloroform. Some effective conditions on the formation of complexes such as pH, shaking time, organic solvent, the concentration of dye were systematically studied. The present method was also applied to determine FQs in some pharmaceutical formulations including tablets, and infusions.

2.EXPERIMENTAL

2.1. Apparatus

A double beam UV-Visible spectrophotometer (SP-60, Biochrom Ltd., UK) with 1.0 cm of path lengthquartz cells was used to measure all absorbance samples. Inolab pH-meter instrument

(Germany) was used to monitor the pH of solutions. Three standard buffers were used to calibrate the electrode before measuring pH of solutions. All measurements were conducted at 25 ± 2 ^{0}C controlled by air conditional laboratory.

2.2. Materials and reagents

All chemicals used were of analytical grade and double distilled water was used to prepare all solutions in the present study.

FQs were purchased from Sigma (Germany, with purity >99.0%), while bromothymol blue (BTB) was supplied from Maya - R, China, with purity > 99%. The organic solvents chloroform, dichloromethane, carbon tetrachloride, dichloroethane, benzene, toluene and other chemicals are analytical reagents (AR, Merck, Germany).

The following dosage forms containing FQs were purchased from local pharmacy market and employed in the study: Hasancip and Kacipro tablets equivalent to 500 mg ciprofloxacin (Hasan-Dermapharm and Dong Nam manufacturing – Trading pharmaceutical Co., Ltd, Viet Nam). Ciprofloxacin infusion equivalent to 200 mg ciprofloxacin /100 ml solution for infusion (Hebei Tiancheng Pharmaceutical Co., Ltd and Shandong Hualu Pharmaceutical Co., Ltd, China). Stada and DHG tablets equivalent to 500 mg levofloxacin (Stada-VN J.V.Company and DHG pharmaceutical joint – stock company, Viet Nam). Ofloxacin (200 mg/tablet) were provided by the Mekophar Chemical Pharmaceutical Company (Viet Nam).

2.3. Solution preparation

A stock solution of FQs (1mg/mL) in double distilled water. The working standard solution of FQs containing 100µg/mL was prepared by appropriate dilution. The stock solution of BTB (0.025%) was prepared in doubly distilled water. All stock solutions were kept in dark bottle, stored in 4^{0}C and could be used within one week.

2.4. Construction of calibration curves

A series of 125 mL separating funnel, the volumes of working solutions of the drugs in different concentration range (CFX (1–35 µg/mL), LFX (0.5–25 µg/mL), OFX (0.5–25 µg/mL) were transferred. Then, adding 4.0mL of 0.025% BTB solution before thoroughly mixing. After that, a 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 yellow colored chloroform layer containing the ion-pair complexes were measured at 420 nm for CFX, 415 nm for LFX and 418 nm for OFX against the reagent blanks. The colored chromogen complexes are stable for 24h.

2.5. Sample preparation

Weigh and mix the contents of twenty tablets each drug (CFX, LFX and OFX), an accurately weighed amount of powder equivalent to 0.1g of drugs transferred into a 100 mL beaker. A magnetic stirrer was used to completely disintegrate the powder in doubly distilled water. Then, filtered through a Whatman paper (No 40) and filled up to 100 mL with doubly distilled water in a volumetric flask. The working solution of the drugs containing 100 µg/mL was prepared by dilution and determined under optimum conditions.

3. RESULTS AND DISCUSSION

3.1. Optimum reaction conditions

3.1.1. Effect of extracting solvent

Six organic solvents including chloroform, carbon tetrachloride, dichloromethane, dichloroethane, benzene and toluene were used to study the effect of solvent to ion pair formation between FQs and BTB. Figure 2 shows that chloroform is the most suitable solvent for extraction of three FQs with low blank absorbance, highest absorbances and lowest standard deviations. It implies that chloroform is the best extracting solvent to achieve a quantitative recovery of the complex with the shortest time to reach the equilibrium processes.

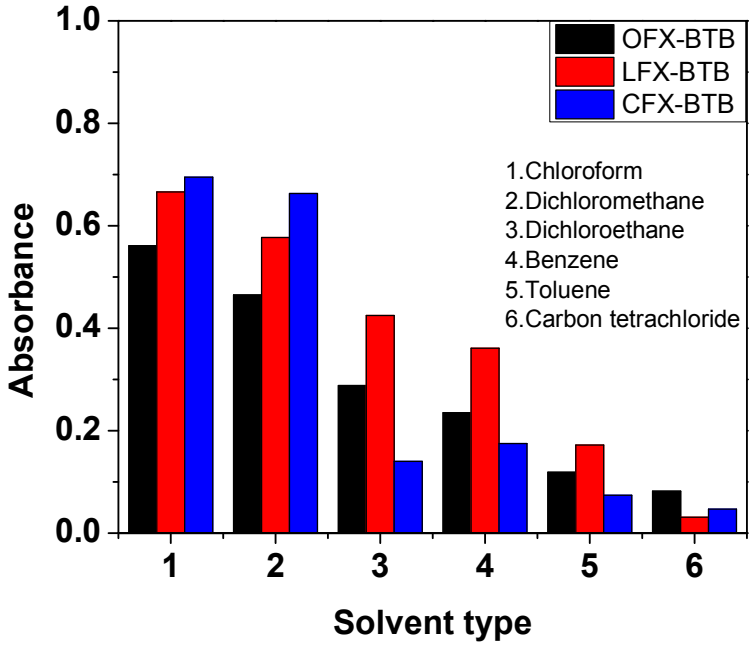
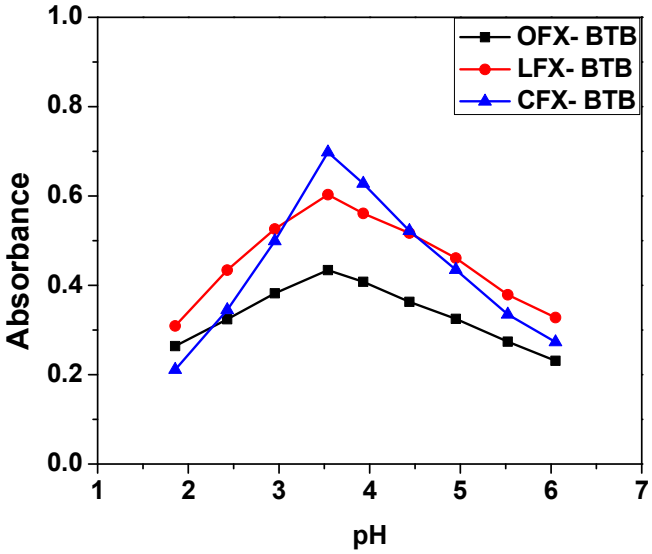


Figure 2: The effect solvent on the ion-pair complex formation (10 µg/mL of fluoroquinolones (FQs) with bromothymol blue (BTB)).

3.1.2. Effect of pH

The pH of solution plays important role in the complexes. The effect of pH on the formation of ion-pairs was examined by varying the pH from 2.0 to 6.0 adjusting by 1 M HCl and 1M NaOH. The maximum absorbances were observed at pH 3.3, 3.4 and 3.5 for the complexes of BTB and OFX, CFX and LFX, respectively (Fig. 3). At these pH values correspond to the initial pH of the examined drug and the dye. Therefore, it is not necessary to adjust the pH before extraction.



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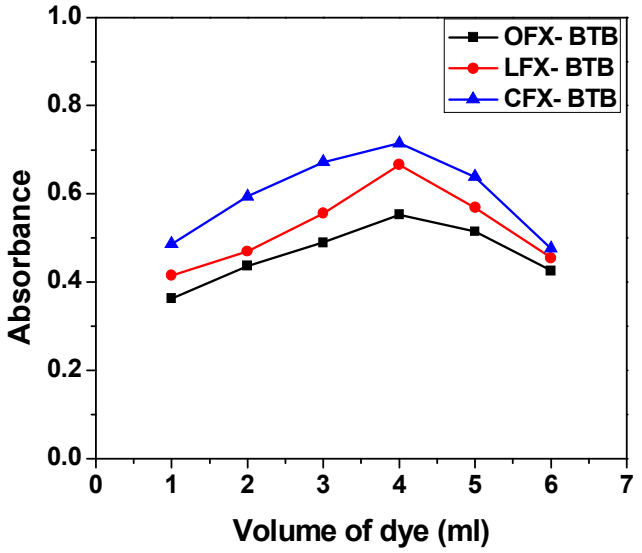
149 *Figure 3:* Effect of pH on the absorbances of 10 µg/mL of OFX, LFX and CFX.

150 *3.1.3. Effect of dye concentration*

151 The effect of dye concentrations was studied by adding different volumes of 0.025 % BTB
152 from 1.0 to 6.0 mL with a fixed concentration of FQs (10 µg/mL) (Fig.4). Figure 4 shows that the
153 maximum absorbance of the complex was achieved with 4.0 mL of 0.025% of BTB in each case
154 and excess dye did not affect the absorbance of the complex. Therefore, 4.0 mL of 0.025% of BTB
155 is optimum dye volume and it is kept as constant for further studies.



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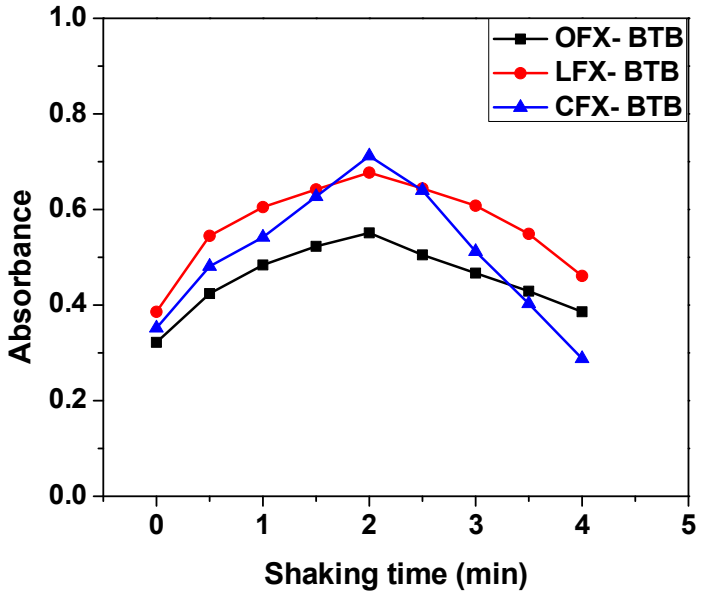
157 *Figure 4:* Effect of the volume of 0.025% BTB to absorbance of 10 µg/mL of OFX, LFX and CFX.

158 *3.1.4. Effect of shaking time*

159 The effect of shaking time on the formation and stability of the ion-pair complex was
160 investigated by measuring the absorbance of the extracted ion-associates with increasing time from
161 0 to 4.0 min. Figure 5 shows that the ion-pair complexes were formed instantaneously with 2.0
162 min shaking time. Thus, 2.0 min is optimum shaking time and it is fixed for further studies.



163

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

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166 *3.1.5. Stoichiometry of ion-pair complexes*

167 Job's method of continuous variation of equimolar solutions was employed to evaluate
168 stoichiometry of the complex. A 3.0×10^{-4}M standard solution of three FQs and 3.0×10^{-4}M
169 solution of BTB were used. A series of solutions was prepared in which the total volume of drug
170 and reagent was kept in 10 mL while the absorbances were measured at 420, 415 and 418 nm, for
171 CFX, LFX and OFX, respectively. The absorbances were plotted against the mole fraction of the
172 drugs. The stoichiometry for each drug–dye ion-pair complex was found to be 1:1 (Fig. 6).

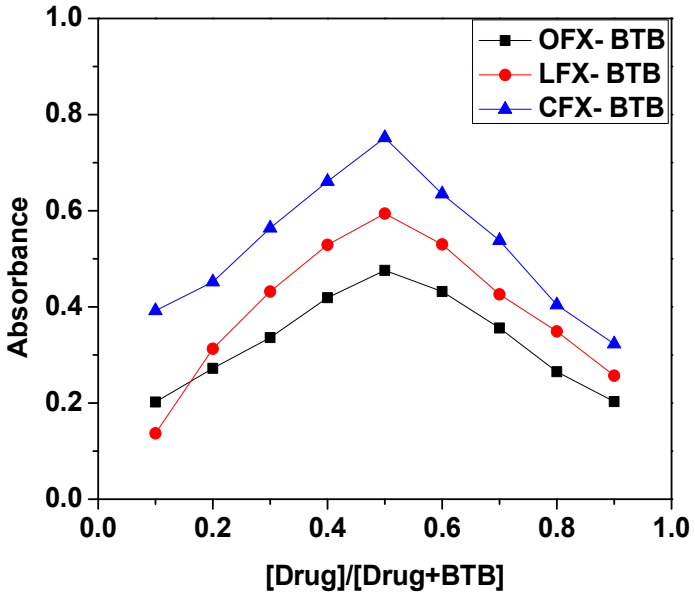

Figure 6: Job's method of continuous variation graph for the reaction of drug with acid-dyes BTB, [drug]=[dye]=3.0×10^{-4}M

3.1.6. Mechanism of reaction and absorption spectra

Fluoroquinolones can contain a secondary amino group (CFX) and tertiary amino group (LFX and OFX), that can be easily protonated under acidic conditions. On the one hand, the sulphonic acid group in BTB that is the only group undergoing dissociation in the pH range 1-5. The colour of BTB is on basis of lactoid ring and subsequent formation of quinoid group. It is suggested that the two tautomers are plausible in equilibrium due to strong acidic nature of the sulphonic acid group. Thus, the quinoid body must predominate. Finally, the protonated fluoroquinolones forms ion-pairs with BTB dye that could be quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in a scheme in Fig. 7.

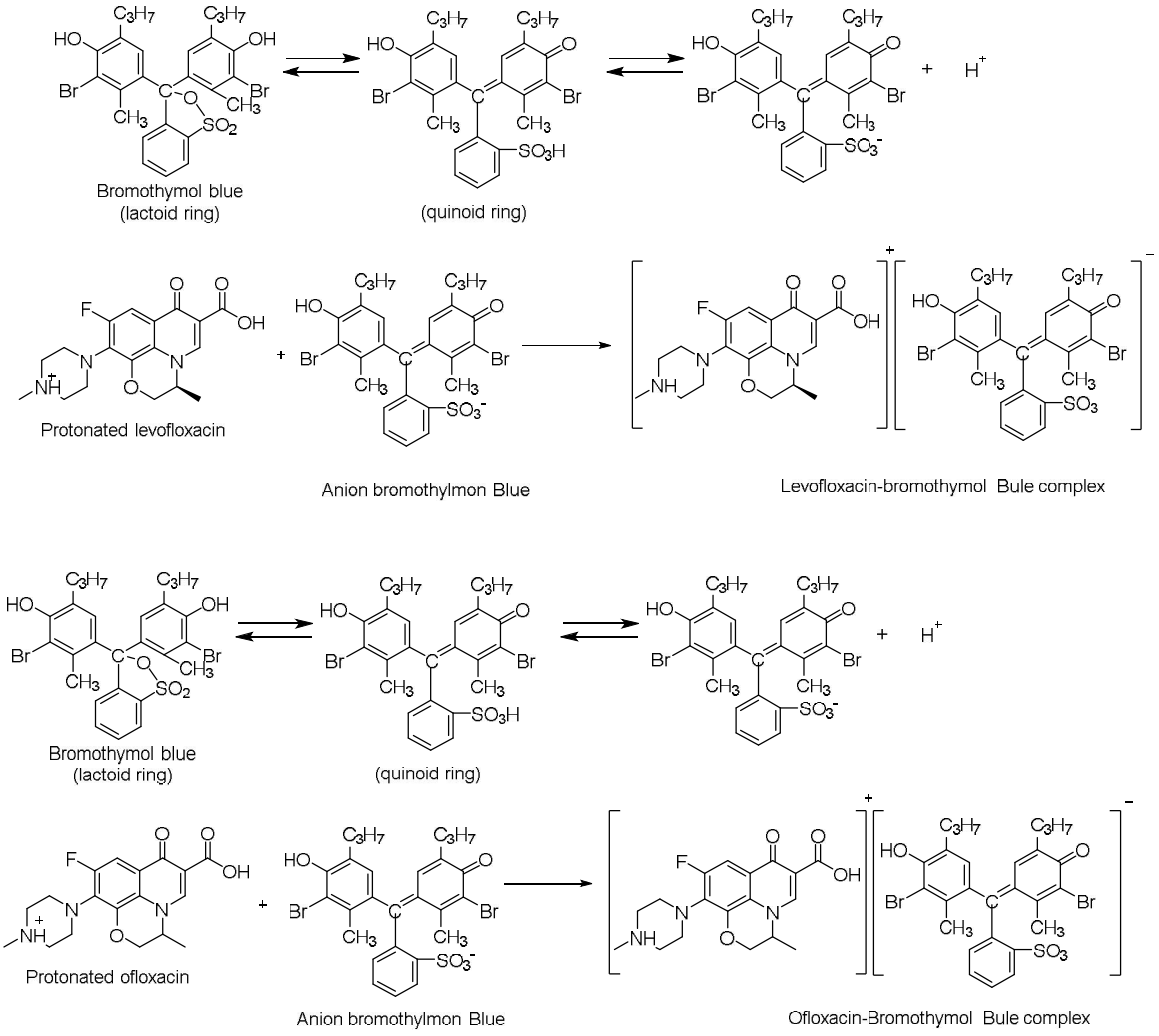

Figure 7: Proposal mechanism for the reaction between levofloxacin, ofloxacin and bromothymol blue

The absorption spectra of the ion-pair complexes, which were formed between FQs and BTB were measured in the wavelength range 350-500nm against the blank solution and shown in Fig. 8.

Figure 8 shows absorption maxima for CFX-BTB, LFX-BTB and OFX-BTB in chloroform were observed at 420, 415, and 418 nm, respectively. The reagent blanks under similar conditions have insignificantly absorbances.

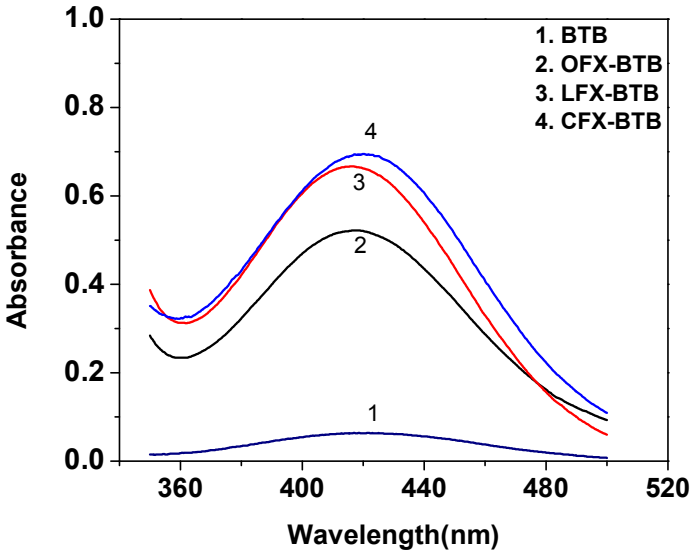

Figure 8: Absorption spectrum of ion-associate complexes of fluoroquinolones (10 µg/mL) with BTB against reagent blank.

3.1.7. Association constants of ion-pair complexes

The equation of association constant of ion-pair complex is

$$\frac{A/A_m}{[1-\frac{A}{A_m}]^{n+2}C_{M(n)n}} \quad (1)$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry in which BTB ion associates with drugs. The conditional stability constants (K_f) of the ion-pair complexes for FQs were calculated from the continuous variation data using the following equation [26]:

$$K_f = \frac{A / A_m}{[1 - A / A_m]^{n+2} C_M (n)^n} \quad (2)$$

The conditional stability constants (K_f) of the ion-pair complexes for FQs are indicated in Table 1.

216 ***Table 1. The conditional stability constants (K_f) of the ion-pair complexes for FQs.***

Sample	V_{drug} (mL)	V_{BTB} (mL)	A	n	n^n	$[1-\frac{A}{A_m}]^{n+2}$	K_f	logK_f	Mean
Ofloxacin									
1	0.25	2.25	0.202	0.1111	0.7834	0.3116	50204.7802	4.7007	**6.08**
2	0.5	2	0.272	0.2500	0.7071	0.1486	157048.9127	5.1960	
3	0.75	1.75	0.336	0.4286	0.6955	0.0512	572507.7789	5.7578	
4	1	1.5	0.419	0.6667	0.7631	0.0035	9562329.8320	6.9806	
5	1.25	1.25	0.476	1.0000	1.0000	0.0000	_	_	
6	1.5	1	0.432	1.5000	1.8371	0.0002	59414177.6247	7.7739	
7	1.75	0.75	0.356	2.3333	7.2213	0.0026	1172246.1806	6.0690	
Levofloxacin									
1	0.25	2.25	0.137	0.1111	0.7834	0.5749	14789.8587	4.1700	**6.04**
2	0.5	2	0.313	0.2500	0.7071	0.1856	115961.3839	5.0643	
3	0.75	1.75	0.432	0.4286	0.6955	0.0426	708574.1920	5.8504	
4	1	1.5	0.559	0.6667	0.7631	0.0005	67745245.6475	7.8309	
5	1.25	1.25	0.594	1.0000	1.0000	0.0000	_	_	
6	1.5	1	0.53	1.5000	1.8371	0.0004	34165312.8945	7.5336	
7	1.75	0.75	0.426	2.3333	7.2213	0.0042	682897.0665	5.8344	
Ciprofloxacin									
1	0.25	2.25	0.392	0.1111	0.7834	0.2112	83530.0520	4.9218	**5.91**
2	0.5	2	0.452	0.2500	0.7071	0.1265	178145.0100	5.2508	
3	0.75	1.75	0.564	0.4286	0.6955	0.0345	828480.0305	5.9183	
4	1	1.5	0.661	0.6667	0.7631	0.0036	8522213.4055	6.9306	
5	1.25	1.25	0.752	1.0000	1.0000	0.0000	_	_	
6	1.5	1	0.615	1.5000	1.8371	0.0026	4572267.1248	6.6601	
7	1.75	0.75	0.538	2.3333	7.2213	0.0043	608798.6008	5.7845	

217 "_": Not determination

Table 1 show that the logK_f values of ion-pair associates for OFX-BTB LFX-BTB and CFX-BTB were 6.08±0.46, 6.04±0.58 and 5.91±0.32, respectively (numbers of replicated experiments n = 6). The obtained results confirmed that the ion-pair formation complexes are high stability.

3.2. Validation of the present method

The proposed methods are validated according to International Conference on Harmonization (ICH) guidelines in respect to linearity, sensitivity, LOD and LOQ, accuracy, precision [27]. The parameters that have been investigated and indicated below.

3.2.1. Linearity, Sensitivity, Limits of Detection and Quantification

A linear between the measured absorbance and the concentration range studied for each drug as shown in Fig. 9, and the correlation coefficients (R) of at least 0.997 were achieved. 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, where SD is the standard deviation of blank absorbance values, b is the slope of the calibration curve equation.

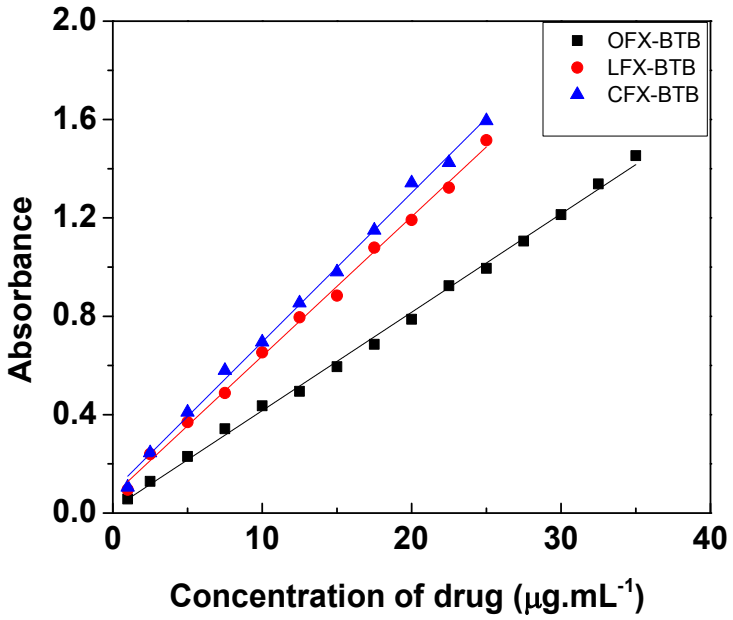

Figure 9: Calibration curves for OFX, LFX and CFX at 418, 415 and 420 nm, respectively.

The LOD and LOQ values, slope and intercept of linear graphs for all the drugs and analytical parameters are indicated in Table 2. The molar absorptivities, Sandell's sensitivity of each methods was calculated and these values showed that the molar absorbtivity of CFX-BTB> LFX-BTB > OFX-BTB ion-pair complexes.

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

Parameters	Proposed methods		
	Ofloxacin	Levofloxacin	Ciprofloxacin
Colour	Yellow	Yellow	Yellow
Wavelengths λ_{max} (nm)	418	415	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 (µg/mL)	1 – 35	0.5 – 25	0.5 – 25
Limit of detection, LOD (µg/mL)	0.105	0.101	0.084
Limit of quantitation, LOQ (µg/mL)	0.315	0.303	0.252
Molar absorptivity (L/mol.cm)	$1.44x10^4$	$2.07x10^4$	$2.09x10^4$
Sandell's sensitivity (µg/cm^2)	0.068	0.048	0.046
Regression equation (Y = bx +a), where Y is the absorbance, a is the intercept, b is the slope and x is the concentration in µg/mL			
Slope (b)	0.040	0.057	0.061
Intercept (a)	0.0165	0.072	0.089
Correlation coefficient (R)	0.998	0.997	0.998

3.2.2. Accuracy and Precision

The accuracy and precision of the methods were determined by preparing solutions of three different concentrations of drug and analyzing them in six replicates. 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.

$$RE\ (\%) = [\frac{(founded - added)}{added}]x100 \quad (3)$$

The accuracy and precision were summarized in Table 3. The low values of the RSD and RE confirm the high precision and the good accuracy of the present method.

252 ***Table 3. Evaluation of accuracy and precision of the proposed methods (n=6)***

Method	Additive concentration (μg/mL)	Found concentration (μg/mL)	Recovery (%)	RSD (%)	RE (%)
Ofloxacin	5.00	5.11	102.19	2.31	2.2
	10.00	10.26	102.64	1.34	2.6
	15.00	14.89	99.25	0.88	-0.73
Levofloxacin	5.00	5.16	102.70	2.03	2.8
	10.00	10.16	101.56	1.10	1.6
	15.00	14.82	98.80	0.50	-1.2
Ciprofloxacin	5.00	5.13	102.71	1.92	2.6
	10.00	9.74	97.41	0.52	-2.6
	15.00	14.60	97.33	0.57	-2.7

253 *3.2.3. Selectivity and effect of Interferences*

254 The effect of commonly utilized excipients in drug formulation was studied. The investigated FQs were
255 studied with various excipients such as magnesium stearate, glucose, lactose, starch and sodium chloride
256 which were prepared in the proportion corresponding to their amounts in the real drugs with final dosage
257 of 10 μg/mL FQ. The effect of excipients on the determination of FQs were evaluated by recovery when
258 determining FQs analyzed with the proposed method in the present of excipient (Table 4).

259 ***Table 4. The effect of excipients on the determination of fluoroquinolones (10 μg/mL)***

Recovery (%) ±SD				
Excipients	Amount excipient added (μg/mL)	Ofloxacin	Levofloxacin	Ciprofloxacin
Magnesium stearate	500	102.04±0.12	101.23±0.089	98.53±0.91
Glucose	250	100.17±0.16	99.04±0.14	99.08±0.062
Lactose	500	99.92±0.21	100.20±0.12	99.73±0.21
Starch	200	100.96±0.24	98.89±0.13	101.31±0.17
Sodium chloride	500	100.13±0.24	100.15±0.11	99.75±0.16

The results in Fig. 4 show that the recoveries are in the range of 98.53 – 102.04, demonstrating that no interference of excipients when FQs in drugs are quantified by extractive spectrophotometric using ion-pair formation with BTB. In other word, the present method has a high selectivity for determining FQs in its dosage forms.

3.3. Comparison with other spectrophotometric methods

The proposed method compares with other reported methods. It has been observed that, the extractive spectrophotometric method with BTB in the present research is more high sensitivity than other ones (Table 5). It also doesnot need heating, the product is stable for a longer time and the interference are minimum.

Table 5. The comparison of present study with other spectrophotometric method

Drug	Reagent	λ_{max} (nm)	Range of determination (µg/ mL)	Molar absorbitivity (L/mol·cm)	Remarks	Ref.
Ciprofloxacin	Co (II) tetrathiocyanate	623	20-240	8.38×10^2	Less sensitive	[28]
	Supracene Violet 3	575	2.5-30	8.62×10^3	Less sensitive	[29]
	Eosin Y	547	2-8	3.56×10^4	Less stable colour	[30]
	Merbromin	545	2-15	1.23×10^4	Addition of CN^- to inhibit Hg^{+2} ions	
	Ce(IV)- MBTH	630	10-50	-	Involves shaking time	[17]
	Tris(o-phenanthroline) iron(II)	510	0.04-7.2	3.4×10^4	Involves shaking time and heating	[18]
	Tris (bipyridyl) iron(II)	522	0.05-9	2.95×10^4	Involves shaking time and heating	
	CL	520	16-96	-	Involves shaking time and heating	[16]
	TCNE	335	0.25-15	-	Involves shaking time and heating	
	Sudan II	550	0.8-7.1	5.3×10^4	Narrow linear range	[8]
	Congo Red	517	0.5-6.0	2.83×10^4		
	Gentian Violet	585	0.5-10	2.21×10^4		
	Brilliant Blue G	610	0.5-6.0	2.86×10^4	Narrow linear range and	[31]

					required pH adjustment	
	Bromocresol green	412	1-20	2.28×10^{4}	required pH adjustment	[14]
	BTB	420	0.5-25	$2.09\text{x}10^{4}$	Highly sensitive with wide linear dynamic ranges, no heating, no pH adjustment	This study
Levofloxacin	Chloranilic acid	521	15-250	1.2×10^{3}	Less sensitive	[14]
	Bromocresol green	411	1-20	2.16×10^{4}	Required pH adjustment	
	Eosin Y	547	2-8	4.83×10^{4}	Less stable colour	[30]
	Merbromin	545	2-15	1.58×10^{4}	Addition of $CN^{-}$to inhibit Hg^{+2} ions	
	Cobalt (II) Tetrathiocyanate	623	20-240	-	Less sensitive	[28]
	Bromophenol blue	424	1.85-31.5	1.98×10^{4}	Required pH adjustment	[19]
	Bromocresol green	428	1.85-25	1.82×10^{4}		
	BTB	415	0.5-25	2.07×10^{4}	Highly sensitive with wide linear dynamic ranges, no heating, no pH-adjustment	This study
Ofloxacin	Supracene Violet 3	575	2.5-25	1.09×10^{4}	Less sensitive	[29]
	Tropaeolin 000	485	2.5-30	8.23×10^{2}	Less sensitive	
	Sudan II	560	0.8-8.4	2.97×10^{4}	Narrow linear range	[8]
	Congo Red	530	0.5-5.5	3.29×10^{4}		
	Gentian Violet	575	0.8-11	2.51×10^{4}		
	Bromocresol purple	400	1.0-16.0	2.4×10^{4}	Required pH adjustment	[32]
	Bromocresol green	410	1.0-16.0	1.96×10^{4}	Required pH adjustment	
	Bromophenol Blue	410	5-25	1.03×10^{4}	Required close pH control and involved extraction steps	[20]
	Bromothymol Blue	415	2-15	2.01×10^{4}		
	Bromocresol Purple	410	2-20	1.64×10^{4}		
	Bromothymol Blue	415	1-35	1.44×10^{4}	Highly sensitive with wide linear dynamic ranges, no heating, no pH-adjustment	This study

3.4. Analysis of Pharmaceutical Formulations

The proposed method was applied successfully for determination of studied drugs in the pharmaceutical formulations (tablets, and infusion) and the results are presented in Table 6. Six replicated determinations were measured. Table 6 shows that satisfactory recovery data were obtained and the recovery efficiency varies from 97.41% to 101.20%, indicating high accuracy of the present method in determining real pharmaceutical samples.

Table 6. Determination of the studied drugs in their pharmaceutical preparations using proposed method (n = 6)

Pharmaceutical preparation	Hasancip tablet	Kacipro tablet	Shandong infusion	Hebei infusion	Levofloxacin Stada	Levofloxacin DHG	Ofloxacin Mekopharm
Labeled amount (mg/form)	500 mg /tablet	500 mg /tablet	200 mg/100 mL	200 mg/100 mL	500 mg /tablet	500 mg /tablet	200 mg /tablet
Recovery (%) ± SD	98.89±0.23	101.20±0.20	97.41±0.42	97.69±0.36	99.53±0.17	101.01±0.35	99.58±0.46

4. CONCLUSIONS

We have reported a new method when using BTB as an anionic dyes for the extractive spectrophotometric determination of ciprofloxacin (CFX), levofloxacin (LFX) and ofloxacin (OFX) in different pharmaceutical drugs (tablets and infusions). The methods have the advantages of simplicity without heating, pH-adjustment and high sensitivity. The limit of detection (LOD) of 0.084 µg/mL for CFX, 0.101 µg/mL for LFX, and 0.105 µg/mL for OFX. No interference from common excipients was confirmed. The stoichiometry complexes of FQs and BTB determined by Job's method of continuous variation was found to be 1:1. The developed and validated methods are indicated the acceptable precision and accuracy, and recovery of the drugs and suitable for routine analysis of drugs in pharmaceutical formulations.

Competing Interests

The authors declare that they have no competing interests.

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Data availability

(1) The data is all carried out at our laboratories at Faculty of Physics and chemical Engineering, Le Quy Don Technical University, 236 Hoang Quoc Viet, Hanoi, Viet Nam and Faculty of Chemistry, VNU- University of Science, Vietnam National University, Hanoi, 19 Le Thanh Tong, Hoan Kiem, Hanoi 10000, Vietnam

(2) The data in the manuscript can be accessed at Faculty of Physics and chemical Engineering, Le Quy Don Technical University, 236 Hoang Quoc Viet, Hanoi, Viet Nam

And Faculty of Chemistry, VNU- University of Science, Vietnam National University, Hanoi, 19 Le Thanh Tong, Hoan Kiem, Hanoi 10000, Vietnam

(3) Some restrictions on data access due to the lack of connection between two above faculties. Two faculty belongs to the different kinds of universities.

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