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Spectrophotometric determination of enoxacin as ion-pairs with bromophenol blue and bromocresol purple in bulk and pharmaceutical dosage form☆

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Abstract

Three simple, accurate and sensitive spectrophotometric methods were developed for determination of enoxacin. The methods based on extraction of this drug into chloroform as ion pairs with sulphonphthalein dyes as bromophenol blue and bromocresol purple. The optimum conditions of the reactions were studied and optimized. The absorbance of yellow products was measured at 412 nm for enoxacin–bromophenol blue and 410 nm for enoxacin–bromocresol purple. Linearity ranges were found to be 2.0–20.0 μg ml^{-1} for enoxacin–bromophenol blue and 0.77–17.62 μg ml^{-1} for enoxacin–bromocresol purple. The detection limits were found to be 0.084 μg ml^{-1} and 0.193 μg ml^{-1} for enoxacin–bromophenol blue and enoxacin–bromocresol purple, respectively. The composition of the ion pairs was found 1:1 by Job's method. The developed methods were applied successfully for the determination of this drug in pharmaceutical preparation. The data obtained by developed methods were compared with the spectrophotometric method in literature. No differences were found statistically. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Enoxacin; Sulphonphthalein dyes; Ion-pair extraction; Spectrophotometry

1. Introduction

Enoxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphtyridine-3-carboxylic acid is one of a new generation of fluorinated quinolone structurally related to nalidixic acid (Fig. 1) [1]. This agent is a new broad spectrum antibacterial drug active against most Gram-negative, Gram-positive bacteria and some anaerobes [2]. The mechanism of effect is based on the inhibition of the DNA-gyrase of bacteria and RNA and protein synthesis [3]. They achieve high concentration in most tissue and body fluids [4]. Therefore enoxacin is used in the treatment of systemic infections including urinary tract, respiratory, gastro-intestinal and skin infections [5–

☆ This work was taken from the M. Sc. thesis of İ. Süslü.

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8]. Different methods were reported for the determination of enoxacin in biological fluids and pharmaceutical preparations with spectrophotometry [9–14], spectrofluorometry [15], high-performance liquid chromatography [16–20], polarography [9,21], voltammetry [22,23], potentiometry and conductometry [24] and electrophoresis [25–28]. Microbiological assays have also been described for the determination of this agent [29,30].

Several methods have been published for the quantitative determination of basic nitrogenous compounds, in which the acid dyes form color compounds extractable from the aqueous solution with organic solvents as chloroform and dichloromethane. The color chromogens in the organic solvents are measured spectrophotometrically.

The aim of this work was to develop spectrophotometric methods for the determination of enoxacin which were based on the formation of ion pairs with bromophenol blue (BPB) and bromocresol purple (BCP) into chloroform phase, then to show the application of the method to pharmaceutical preparations.

2. Experimental

2.1. Apparatus

A Shimadzu UV-160 A recording double beam UV–visible spectrophotometer with data processing system was used. UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 50 nm min^{-1} and fixed slit width of 3 nm. An Orion 100-Nüve pH-meter with combined glass saturated calomel electrode was used for pH measurements.

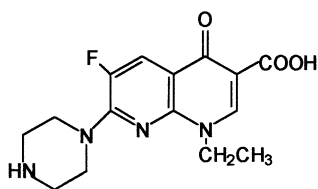


Fig. 1. Chemical structure of enoxacin.

2.2. Reagents and solutions

All chemicals and reagents were of analytical grade and water was always doubly distilled.

Enoxacin was supplied by Eczacıbaşı A.Ş. Purity of this substance was tested by controlling its melting point, UV and IR spectra. No impurities were found. All other chemicals were obtained from Merck. The film coating Enoxetin tablets were obtained from Eczacıbaşı A.Ş. containing 400 mg enoxacin per tablet.

Standard of enoxacin (1 mg ml^{-1}) was prepared by dissolving pure drug in 0.5 N acetic acid for enoxacin-BPB method. The other 10^{-3} M enoxacin standard solution (32.03 mg/100 ml), was prepared by dissolving pure drug in 3 ml 0.5 N acetic acid and completed to 100 ml with water for enoxacin–BCP method.

A stock solution of BPB was prepared by dissolving 100 mg in Mc-ilvaine citrate buffer pH 4.0 and completed to 100 ml with same buffer.

A stock solution of BCP was prepared by dissolving 162 mg in 20 ml% 96 ethanol and diluted to 100 ml with water.

Mc-ilvaine citrate buffer (pH 4.0 for enoxacin–BPB) was prepared by dissolving 21.0 g citric acid in 200 ml 0.1 M sodium hydroxide and completed to 1000 ml with water and adjusting pH by addition of 0.2 M disodium hydrogen phosphate.

Potassium hydrogen phthalate buffer (0.1 M, pH 3.3 for enoxacin–BCP) was prepared by dissolving 1.020 g potassium hydrogen phthalate in water and completed to 50 ml with water and adjusting pH by addition of 0.1 M hydrochloric acid.

2.3. Procedure

2.3.1. Enoxacin–bromophenol blue method

Aliquots of 0.1–1.0 ml, standard drug solutions (1 mg ml^{-1} enoxacin) were transferred to stoppered flasks and 1 ml BPB solution in buffer was added in each flask. The reaction mixture was extracted with 5 ml chloroform by shaking for 1 min at vortex. The yellow colored organic layer was taken and measured the absorbance at 412 nm against the corresponding blank solution. The reagent blank solution was prepared in the same

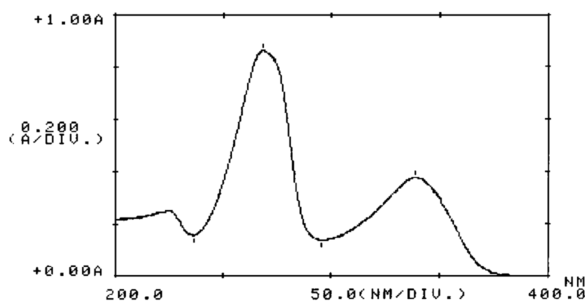


Fig. 2. Absorption spectra of 8 μg ml^{-1} of enoxacin solution in 0.5 N acetic acid.

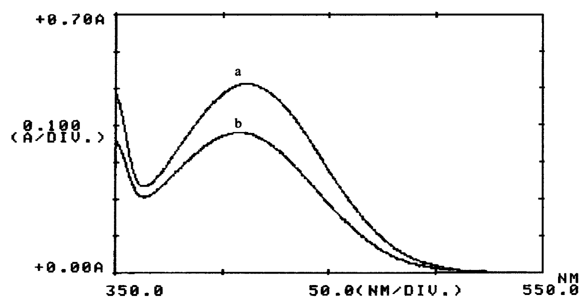


Fig. 3. Absorption spectra of (a) 10 μg ml^{-1} of enoxacin–BPB (b) 6.41 μg ml^{-1} enoxacin–BCP ion-pair complex in chloroform.

manner without enoxacin. All measurements were made at room temperature (25 ± 1 °C).

2.3.2. Enoxacin–bromocresol purple method

Aliquots of 0.12–2.75 ml, standard drug solutions (320.3 μg ml^{-1} enoxacin) were transferred to stoppered flasks and added 1 ml potassium hydrogen phthalate buffers in the case of pH 3.3 and 5 ml of BCP. The reaction mixture was extracted with 5 ml chloroform by shaking for 1 min at vortex. 1 ml organic layer was completed to 10 ml with chloroform in volumetric flasks. The absorbance of yellow colored chloroformic extracts was measured at 410 nm against the corresponding blank solution. The reagent blank solution was prepared in the same manner without enoxacin. All measurements were made at room temperature (25 ± 1 °C).

2.3.3. Sample preparation

Twenty tablets were weighed and powdered. An amount equivalent to one tablet was extracted with 25 ml 0.5 N acetic acid four times and filtered. Necessary amounts of filtrate were diluted to 100 ml acetic acid and the same procedure were applied as described under the procedure for bulk samples (Sections 2.3.1 and 2.3.2).

3. Results and discussion

Several extractive spectrophotometric methods have been published for the basic nitrogenous compounds, in which the acidic dyes form to yield ion-pair salts that color compounds extractable from the aqueous solution to organic phase in literature [31,32]. As shown in Fig. 1, enoxacin is an amino compound containing piperazine ring. Ion-pairs formed between enoxacin and sulphonphthalein dyes can be used for its determination in bulk and pharmaceutical forms. In this study two spectrophotometric methods were developed based on the extraction of ion-pairs of enoxacin with BPB and BCP into chloroform for the determination of enoxacin. Then the linearity, accuracy, precision, sensitivity, stability of proposed methods were described and applied to pharmaceutical preparation as tablet and obtained results evaluated statistically.

3.1. Optimization of the analytical procedures

In proposed methods, some variables in the reaction conditions were studied and the influence of these variables on the reaction was tested.

3.1.1. Selecting of the wavelengths

Pure enoxacin in 0.5 N acetic acid was determined by direct spectrophotometry and the method applied to the analysis of this drug in tablet form. Absorption spectra of enoxacin were obtained at 200–400 nm against blank solution. In this spectrum, enoxacin shows two maximum peaks at 268 and 338 nm (Fig. 2). Analysis was made at 338 nm.

The absorption spectra of the ion-pair complexes, formed between enoxacin and each of BPB

and BCP were measured at 350–550 nm against the blank solution and shown in Fig. 3. The yellow chloroformic extracts show maximum absorbance at 412 nm for enoxacin–BPB and at 410 nm for enoxacin–BCP methods. The measurements were made at these wavelengths for bulk and tablet samples.

3.1.2. Selecting of the extracting solvents

The effect of the extracting solvent on the ion-pair complexes was examined. Two organic solvents as dichloromethane and chloroform were studied and chloroform was selected because of its slightly higher efficiency on color intensity, selective extraction of the drug–dye complex from the aqueous phase and obtained highest absorbance with chloroform.

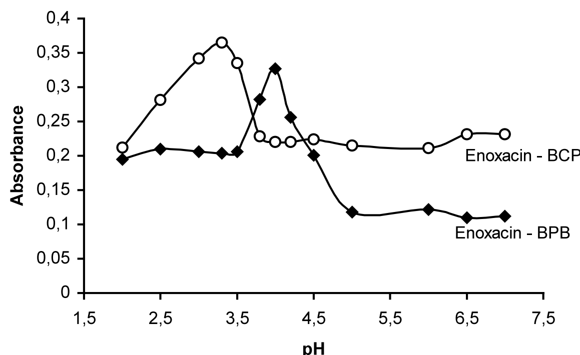


Fig. 4. Effect of pH of buffer solution on the ion-pair complexes.

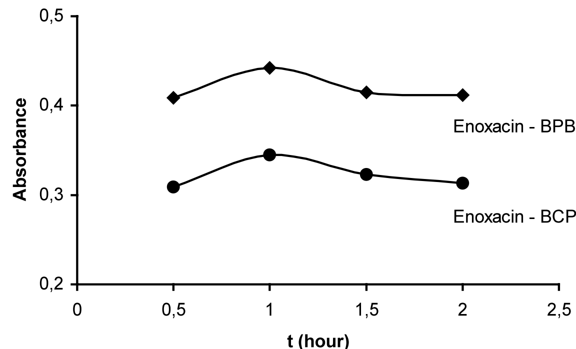


Fig. 5. Effect of shaking time on the ion-pair complexes.

3.1.3. Effects of pH buffer

The effect of pH of buffer solutions on the ion-pairs was studied. Different pH (2–7) values were tested and the absorbance reading of the drug–dye complexes were examined. pH 4.0 Mcilvaine citrate buffer for enoxacin–BPB and pH 3.3 potassium hydrogen phthalate buffer for enoxacin–BCP were found the optimum buffers. Maximum absorbances were achieved at these buffers for studied ion-pair complexes (Fig. 4).

3.1.4. Dye concentration

The effect of the dye concentration on the intensity of the color developed at selected wavelengths was tested using different milliliters of the reagent. The results shown that 1 ml of BPB and 5 ml of BCP were found to be optimum for these proposed methods and excess of these dyes do not affect the color of the complex or the absorbance.

3.1.5. Selected shaking form and times

Ion-pair complexes were formed by using magnetic stirrer, vortex and separating funnel and maximum absorbances were achieved in vortex. Shaking times ranging from 0.5 to 2 min were studied and the optimum shaking time at 1 min was selected (Fig. 5).

3.1.6. Stoichiometric relationship

The composition of the ion pairs associates was established by Job's method of continuous variation and mole-ratio methods [33]. In these methods enoxacin, BPB and BCP were prepared in the same concentration (10^{-3} M). Different amounts of enoxacin and dyes were added to each flask and extracted in the same manner as in Sections 2.3.1 and 2.3.2 for Job's and mole-ratio method. The reagent blank solution without enoxacin was prepared in Sections 2.3.1 and 2.3.2. The absorbances of formed ion-pair complexes were measured at 412 for enoxacin–BPB and 410 nm for enoxacin–BCP. The absorbance was plotted against [enoxacin]/[enoxacin] + [dye] for Job's method and [dye]/[enoxacin] for mole-ratio method. The results obtained showed that 1:1 complexes were formed by these proposed methods (Figs. 6 and 7). The suggested mechanisms of enoxacin–BPB and enoxacin–BCP ion-pair complex formation are described in Schemes 1 and 2.

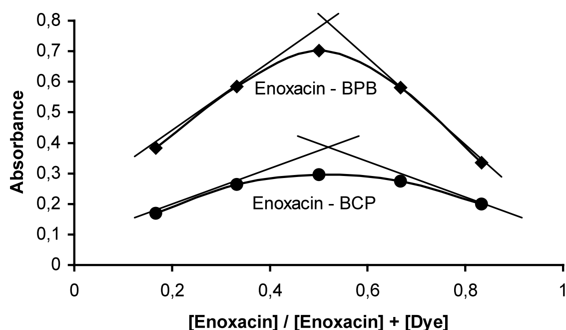


Fig. 6. Continuous variation plots for enoxacin–BPB and enoxacin–BCP in chloroform (1×10^{-3} M).

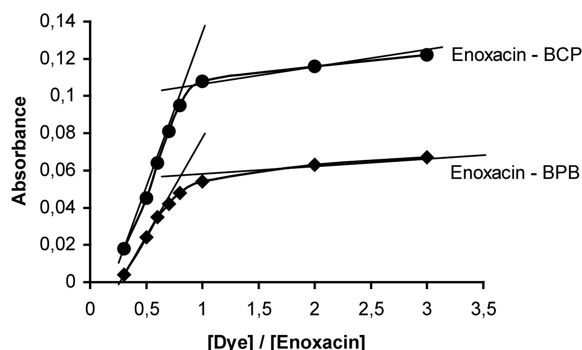


Fig. 7. Mole-ratio plots for enoxacin–BPB and enoxacin–BCP in chloroform (1×10^{-3} M).

3.1.7. Stability of ion-pair complexes

The stability of the ion-pair complexes formed between drug and acidic dye was evaluated. The formation of the ion-pairs were rapid and the yellow color extracts were stable for 2 h for enoxacin–BPB and 8 days for enoxacin–BCP without any change in color intensity or in the λ_{max} in dark and room temperature.

3.1.8. Conditional stability constants (K_f) of the ion-pair complexes

The conditional stability constants (K_f) of the ion pair complexes for enoxacin were calculated from the continuous variation data using the following equation:

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

where A and A_m are the observed maximum absorbance and the absorbance value of all the drugs present is associated, respectively. C_M is the molar concentration of drug at the maximum and n is the stoichiometric constant with which dye ion associates with drug. In accordance with the formula, the conditional stability constants were found to be 4.46 ± 0.33, for enoxacin–BPB and 4.99 ± 0.43 for enoxacin–BCP ($n = 5$).

3.1.9. Ionic-strength of the ion-pair complexes

The ionic-strength in the enoxacin–BPB and enoxacin–BCP solutions were calculated. The obtained results were $1.65 \times 10^{-4} \pm 3.08 \times 10^{-6}$ M for enoxacin–BPB, and $1.51 \times 10^{-3} \pm 3.38 \times 10^{-6}$ M for enoxacin–BCP method. The results showed that when the concentration of enoxacin was increased, the magnitude of the ionic-strengths was not almost changed.

4. Method validation

4.1. Linearity of the calibration curves

Under the experimental conditions described, calibration graphs were obtained for each proposed methods. The regression equations, standard errors of slopes and intercepts, determination coefficients, relative standard deviation of response factors and linear ranges were given in Table 1 for each spectrophotometric method. Regression analysis indicated linear relationship between absorbance and concentration; the determination coefficients were 0.9998 which showed good linearity. The calculated molar absorptivity of enoxacin–BCP ion-pair complex was found to be higher than the enoxacin–BPB ion-pair complex.

4.2. Sensitivity

The detection limits (LOD) for the proposed methods were calculated using the following equation [34]:

$$\mathrm{LOD} = 3s/k$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance, were found to be 0.093 μg ml^{-1} for enoxacin at 368 nm, 0.084 μg ml^{-1} for enoxacin–BPB, and 0.193 μg ml^{-1} for enoxacin–BCP methods.

The limits of quantitation, LOQ, defined as [34];

$$LOQ = 10s/k$$

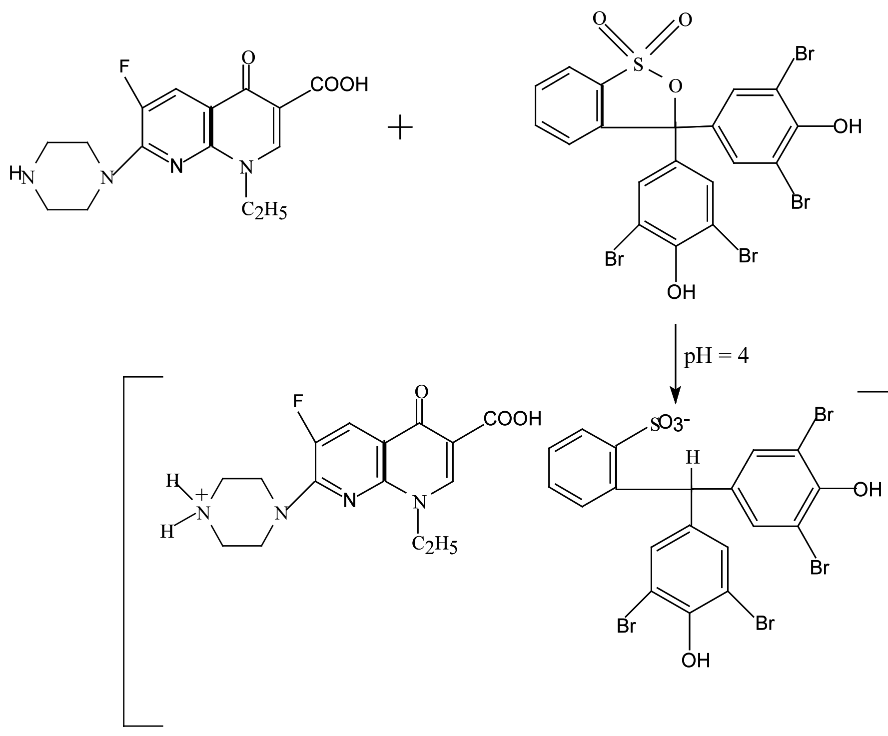
According to this equation, the limits of quantitation were found to be 0.204 μg ml^{-1} for enoxacin at 368 nm, 0.281 μg ml^{-1} for enoxacin–BPB, and 0.644 μg ml^{-1} for enoxacin–BCP methods.

4.3. Specificity, precision, accuracy

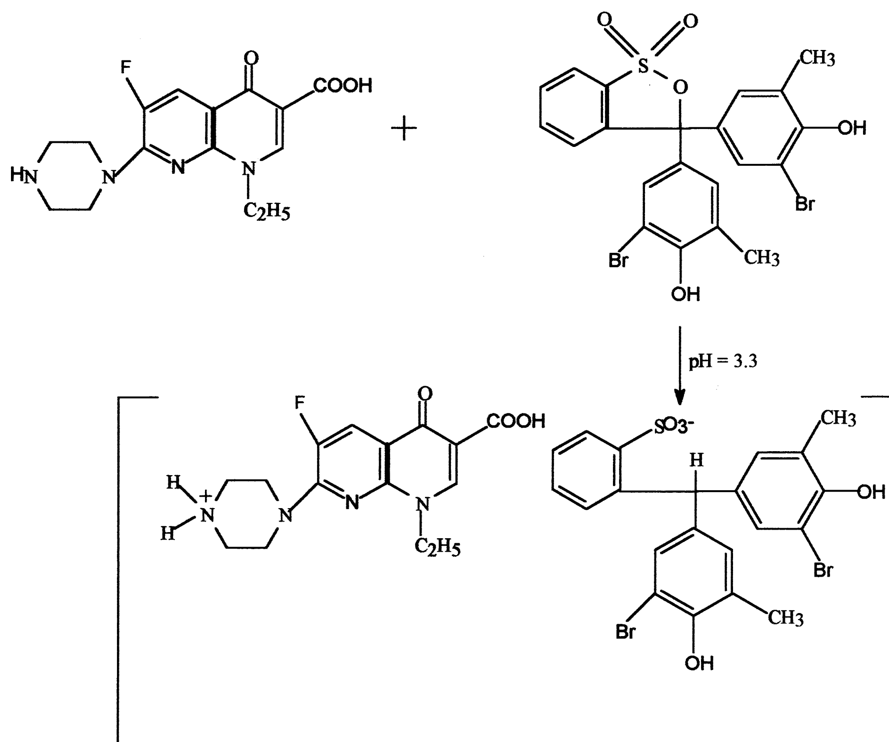
Specificity of ion-pair reaction, selective determination of enoxacin, the basic nitrogenous compounds, with sulphonphthalein dyes could be possible. Precision as percentage relative standard deviation (RSD%) and accuracy as percentage relative error (Er%) of the suggested method were calculated. Precision was carried out six determination at four different concentrations in these spectrophotometric methods. The percentage relative error calculated using the following equation:

$$Er\% = [(\text{founded} - \text{added})/\text{added}] \times 100$$

The inter-day and intra-day precision and accuracy results are shown in Table 2. The results are indicated that good accuracy and precision of the proposed procedures for the analysis of enoxacin.



Scheme 1. Suggested mechanism of enoxacin–BPB ion-pair complex formation.



Scheme 2. Suggested mechanism of enoxacin–BCP ion-pair complex formation.

Table 1
Analytical data and regression characteristic of enoxacin using proposed three spectrophotometric methods ($n = 6$)

Parameters	Enoxacin	Enoxacin–BPB	Enoxacin–BCP
Wavelengths (nm)	338	412	410
Regression equation	$y = 0.0479x + 0.0021$	$y = 0.0431x - 0.0178$	$y = 0.0510x + 0.0026$
Standard error of slope	2.61×10^{-5}	2.23×10^{-4}	3.15×10^{-4}
Standard error of intercept	1.98×10^{-4}	1.12×10^{-3}	4.37×10^{-4}
Determination coefficient (r^2)	0.9998	0.9998	0.9998
RSD of response factor (K)	1.82%	2.16%	2.35%
Linear range (μg ml^{-1})	0.28–40.00	2.00–20.00	0.77–17.62
LOD (μg ml^{-1})	0.093	0.084	0.193
LOQ (μg ml^{-1})	0.204	0.281	0.644
ε (l mol^{-1} cm^{-1})	1.53×10^4	1.31×10^4	1.66×10^4

4.4. Analysis pharmaceutical preparations

The suggested methods were applied to the determination of enoxacin in pharmaceutical preparations. The recoveries calculated as:

$$\%\text{recovery} = (\text{measured enoxacin} \times 100)/\text{added enoxacin}.$$

The values obtained by the proposed methods for tablets are shown in Table 3. Enoksetin tablets

were tested for possible interference in the standard addition method. The regression equations of standard addition curves were found for enoxacin at 338 nm, enoxacin–BPB and enoxacin–BCP methods as $y = 0.0480x + 0.0017$, $y = 0.0426x - 0.0185$, $y = 0.0497x + 0.0056$, re-

Table 2
The inter-day and intra-day precision and accuracy data for enoxacin obtained by the proposed methods ($n = 6$)

Method	Added (μg ml^{-1})	Inter-day			Intra-day		
		Founded[a] (μg ml^{-1})	Precision RSD%	Accuracy Er%	Founded[a] (μg ml^{-1})	Precision RSD%	Accuracy Er%
Enoxacin at 338 nm	1.20	1.16 ± 0.01	2.67	3.17	1.08 ± 1.04	3.53	−10.03
	10.00	9.97 ± 0.04	1.05	−0.26	10.03 ± 0.03	0.63	0.26
	26.00	25.87 ± 0.04	0.31	−0.51	25.94 ± 0.03	0.30	−0.23
	34.00	34.01 ± 0.02	0.14	0.02	34.03 ± 0.04	0.27	0.08
Enoxacin–BBP	2.00	1.94 ± 0.03	3.97	−2.95	1.92 ± 0.03	3.64	−3.95
	10.00	10.12 ± 0.06	1.33	1.23	10.17 ± 0.03	0.75	1.73
	12.00	12.05 ± 0.09	1.82	0.43	12.08 ± 0.09	1.85	0.67
	20.00	20.13 ± 0.16	1.89	0.66	20.21 ± 0.09	1.04	1.06
Enoxacin–BCP	1.60	1.65 ± 0.01	1.95	2.81	1.61 ± 0.02	3.29	0.75
	9.61	9.56 ± 0.09	2.28	−0.55	9.70 ± 0.05	1.37	0.95
	12.81	12.84 ± 0.05	0.92	0.26	12.83 ± 0.05	0.88	0.16
	17.30	17.31 ± 0.05	0.75	0.07	17.29 ± 0.04	0.60	0.06

n, number of determination.
[a] Mean ± standard error; RSD%, percentage relative standard deviation; Er%, percentage relative error.

Table 3
The results of pharmaceutical preparation containing enoxacin analyzed by each spectrophotometric method ($n = 7$)

Sample number	Enoxacin at 338 nm	Enoxacin–BCP	Enoxacin–BPB	Reference method[9]
1	398.84	401.64	401.84	397.60
2	393.84	412.52	398.84	398.60
3	397.44	403.80	401.00	401.55
4	398.12	391.84	385.08	395.65
5	398.12	403.80	392.24	399.60
6	398.84	397.28	397.00	397.60
7	400.28	399.44	400.36	400.60
X	397.93 ± 0.76	401.47 ± 2.43	396.62 ± 2.28	398.74 ± 0.76
SD	2.01	6.42	6.03	2.01
RSD%	0.51%	1.60%	1.52%	0.50%
CI	396.07–399.79	395.53–407.42	391.04–402.20	396.88–400.61
Recovery	99.48 ± 0.19	100.37 ± 0.61	99.16 ± 0.57	99.69 ± 0.19
$F_c = 1.10 \times 10^{-3}$, $F_t = 3.89$ $(P > 0.05)$[a]			$t_c = 6.5$, $t_t = 2$ $(P > 0.05)$[b]	

Labelled to contain 400 mg enoxacin in one tablet of ENOKSETİN tablet. CI, confidence intervals ($\alpha = 0.05$).
[a] F_c = calculated F value, F_t = tabulated F value.
[b] t_c, calculated t value; t_t, tabulated t value.

spectively. There was no significant difference between slopes of three methods with calibration curves and standard addition methods. Therefore it is concluded that there is no spectral interaction in the analysis that the excipients in tablet forms of enoxacin. From the results shown in Table 3, the methods gave satisfactory recovery data. The performance of proposed methods were judged by one-way ANOVA test. At 95% confidence level, the theoretical F values were obtained higher than calculated F values (Table 3). Therefore, there is no significant difference among enoxacin at 338 nm, enoxacin–BPB and enoxacin–BCP methods.

4.5. Comparison of the methods

A spectrophotometric method in literature [9] was employed as a comparison to evaluate the validity of the developed method. The comparison was performed between enoxacin–BPB and reference method, because the proposed method at 338 nm and reference method were made in acidic solution. Table 3 gives the results obtained by enoxacin–BPB method and reference method for the determination of enoxacin in pharmaceutical preparations. The results were compared by the Wilcoxon test and there was no significant difference between the methods at $P > 0.05$. In the range of concentrations studied, 0.28–40.00 μg ml^{-1} for enoxacin at 338 nm, 2.00–20.00 μg ml^{-1} for enoxacin–BPB and 0.77–17.62 μg ml^{-1} for enoxacin–BCP, these proposed methods showed good linearity values and the linearity ranges of the proposed methods were more extensive than the spectrophotometric method in literature (1.60–12.81 μg ml^{-1}) [9].

Ion-pair complex formation was successfully utilized for the determination of fluoroquinolones derivatives. Ciprofloxacin, norfloxacin, ofloxacin, lomefloxacin and enrofloxacin were determined with supracene–violet 3B, tropaeolin 000, BCP, BPB, bromthymol blue, methyl orange as sulphonphthalein dyes [35–39]. Different buffer solvents as phthalate, potassium hydrogen phthalate, phosphate, and acetate buffers were used in these methods. Dichloromethane and chloroform were preferred for their selective extraction of the drug–dye complex from the aqueous phase. The absorbance of colored ion-pair complexes was measured at 410 and 425 nm, but the orange color complex was measured at 575 and 485 nm in supracene–violet 3B and tropaeolin 000 method; the yellow color complex was measured at 350 nm in ceric sulphate/ammonium sulphate method. The colored ion-pair complexes were stable for at least 8 h [36], at 1 h [37], at 24 h [38], at 24 h [39]. When the proposed methods for enoxacin and sulphonphthalein dyes were compared with these methods in literature, the linearity ranges were more extensive for the proposed methods and the enoxacin–BCP ion-pair complex was more stable. Additionally, the LOD and LOQ values for proposed methods were lower than these methods.

5. Conclusion

The proposed methods are simple and rapid. The maximum color development of enoxacin–BPB and enoxacin–BCP ion-pair complex formation is completed immediately after all reagents were added. All the measurements were made in 30 min after the preparation of the solutions in all the experiments. No heating or standing was needed. The developed methods are not time consuming procedures such as the standard addition method and there is also no need for any expensive equipment. These methods do not involve procedural steps, take more operator time and expertise like HPLC and other methods. In the present study, enoxacin was estimated successfully as pure compounds as well as components in representative dosage formulation. The commonly used additives and excipients in pharmaceutical dosage form of enoxacin such as starch, lactose, talc, stearic acid, FD&C Blue No.2 as color substance were found not to interfere in the analysis. The proposed methods are simple and rapid with reasonable precision and accuracy when compared to many of the reported methods. Thus the proposed methods can be employed for the routine determination of enoxacin in bulk samples and pharmaceutical preparations.

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