ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 173–180

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short Communication

Spectrophotometric determination of enrofloxacin and pefloxacin through ion-pair complex formation

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Received 16 February 2001; received in revised form 20 August 2001; accepted 8 September 2001

Abstract

Two simple, quick and sensitive spectrophotometric methods are described for the determination of enrofloxacin and Pefloxacin. The methods are based on the reaction of these drugs with bromophenol blue (BPB) and methyl orange (MO) in buffered aqueous solution at pH 2.3–2.5 in case of bromophenol blue and at pH 3.6 with MO to give highly coloured complex species, extractable with chloroform. The coloured products are quantitated spectrophotometrically at 420 and 424 nm for BPB and MO, respectively. Optimisation of the different experimental conditions is described. Beer's law is obeyed in the concentration ranges 2–12 and 2–18 μg ml^{-1} with BPB and in the ranges 1–12 and 4–40 μg $ml^{-1}$with MO for enrofloxacin and pefloxacin, respectively. The proposed methods are applied for determination of Enroxil oral solution, Peflacine tablets and Peflacine ampoules with mean percentage accuracies 99.5 ± 0.99, 99.39 ± 1.05 and 100.02 ± 0.895, respectively, with BPB and 100.30 ± 0.89, 100.25 ± 0.98 and 100.20 ± 0.72, respectively, with MO.

Keywords: Enrofloxacin; Pefloxacin; Bromophenol blue; Methyl orange; Spectrophotometry; Ion-pair complexes

1. Introduction

Fluoroquinolones are broad-spectrum antibacterial agents, they are effective against most Gram-negative and Gram-positive aerobic bacteria [1]. Enrofloxacin and pefloxacin are members of this group.

Several methods were reported for the determination of these compounds, including spectrophotometry, after formation of complex with Fe(III) [2,3], or with eosin and palladium [4] also through charge-transfer complexation with tetrachloro-benzoquinone, *p*-benzoquinone, *p*-nitrophenol, dichloro-dicyano-*p*-benzoquinone, *p*-chloranil [5–8].

Enrofloxacin was determined spectrophotometrically in its dosage forms through formation of complex with Fe(III), charge transfer complex with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone,

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00591-X

ion-pair complex with bromocresol purple [9]. Pefloxacin was determined titrimetry with benzyl dimethyl alkylammonium bromide [10]. Also chromatographic methods using HPLC and reversed phase HPLC were described for the determination of enrofloxacin and pefloxacin [11–14]. The present study describes Spectrophotometric methods for the determination of enrofloxacin and pefloxacin through ion-pair complex formation with bromophenol blue (BPB) and methyl orange (MO). The reaction conditions and the application of the methods for the determination of enrofloxacin and pefloxacin in their pharmaceutical dosage forms have been established. The proposed methods are simple, quick, economic and provide sensitive procedures compared with other reported spectrophotometric methods.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) 160 IPC UV-visible spectrophotometer connected to an IBM compatible fitted with UVPC Personal spectroscopy software version 3.7 (Shimadz) was used.

2.2. Materials and reagents

All chemicals and reagents were of analytical grade. Absolute alcohol and chloroform (BDH, England), bromophenol blue (Aldrich, England), were prepared as 1 mg ml^{-1} in aqueous ethanol. Methyl orange (Aldrich), was prepared as 1.5 mg ml^{-1} in distilled water. Buffer pH 2.5 was prepared as: 12.5 g of potassium chloride and 1 g of sodium acetate trihydrate were dissolved in 50 ml distilled water, glacial acetic acid was added till a pH 2.5 was obtained and volume was completed to 100 ml with distilled water. Buffer pH 2.3 was prepared as before, adding glacial acetic acid till a pH of 2.3 and the volume completed to 100 ml. Buffer pH 3.6, was prepared by dissolving 12.5 g of KCl and 7 g of sodium acetate trihydrate in 70 ml distilled water, glacial acetic acid was added till a pH 3.6 was obtained and volume was completed to 100 ml with distilled water. Enrofloxacin and pefloxacin mesylate dihydrate, were obtained from (Amriya, Alexandria, Egypt). Standard enrofloxacin solution, was prepared as 0.1 mg ml^{-1} in aqueous solution (for BPB method) and as 0.05 mg ml^{-1} in aqueous solution (for MO method). Standard pefloxacin solution was prepared as 0.1 mg ml^{-1} in distilled water (for BPB method) and as 0.2 mg ml^{-1} in distilled water (for MO method). Enroxil 10% oral solution was obtained from (KRKA, Novomesto, Slovenia), labelled to contain 100 mg enrofloxacin per ml. Peflacine tablets and Peflacine ampoules were manufactured by Amriya, Alexandria, Egypt and labelled to contain 400 mg pefloxacin as pefloxacin mesylate dihydrate per tablet or per ampoule.

2.3. Methods

2.3.1. General procedure

Accurate aliquots containing 0.05–0.3 and 0.025–0.3 mg of enrofloxacin or 0.05–0.45 and 0.1–1 mg of pefloxacin for BPB, MO methods, respectively, were transferred into 25 and 50 ml calibrated flasks for BPB, MO methods, respectively, followed by 4 ml buffer solution pH 2.5 and 2.3 for enrofloxacin and pefloxacin, respectively, for BPB method and pH 3.6 for both drugs with MO method. Then 5 ml of BPB or MO solution was added. The contents were mixed and completed to volume with distilled water. The contents of the calibrated flask were transferred to a separating funnel and extracted with 20 ml chloroform (added in three portions), the collected extract was transferred into 25-ml calibrated flask, then 1 ml absolute ethanol was added and the volume was completed with chloroform. The yellow-coloured chloroformic extract was measured at 420 or 424 nm for BPB and MO, respectively, against a reagent blank prepared in the same manner except addition of drug.

2.3.2. Procedure for Enroxil 10% oral solution

A 0.1 or 0.05 ml of Enroxil 10% oral solution

for BPB and MO methods, respectively, was transferred into a 100 ml calibrated flask, 0.5 ml of glacial acetic acid was added and then diluted to volume with distilled water. Thereafter, the general procedure was followed.

2.3.3. Procedure for Peflacine tablets

An accurately weighed amount of powered tablets equivalent to 10 or 20 mg of pefloxacin for BPB and MO methods, respectively, was dissolved in distilled water, filtered into a 100-ml calibrated flask and diluted to volume with distilled water, then the general procedure was followed.

2.3.4. Procedure for Peflacine ampoules

A 0.125 or 0.25 ml of Peflacin ampoules for BPB and MO methods, respectively, was transferred into a 100-ml calibrated flask, and diluted to volume with distilled water, then the general procedure was followed.

3. Results and discussion

Enrofloxacin and pefloxacin are amino compounds as they contain piperazine moieties, therefore, attempts were made to determine them in aqueous solution by forming extractable salts or ion pairs between these positively charged amino compounds at the proper acidic pH and negatively charged dye or indicator like BPB and MO. The theoretical basis of this method is that the dissociation equilibrium of a BA-type electrolyte dissociating in aqueous medium according to the Eq. (1) can be shifted toward the left (association) if the associate (ion-pair) is removed by extraction by means of a solvent immiscible with water:

$$BA \rightleftharpoons B^+ + A^- \quad (1)$$

where B^+ is the protonated amino drug (enrofloxacin or pefloxacin) and A^- is the BPB or MO anion form.

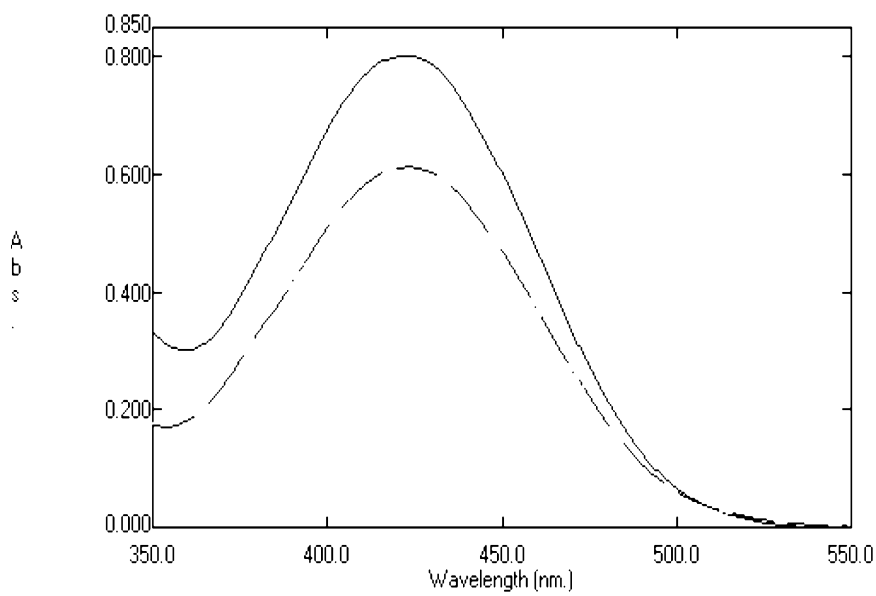


Fig. 1. Absorption curve of enrofloxacin (——) and pefloxacin (— —): bromophenol blue ion pair complexes.

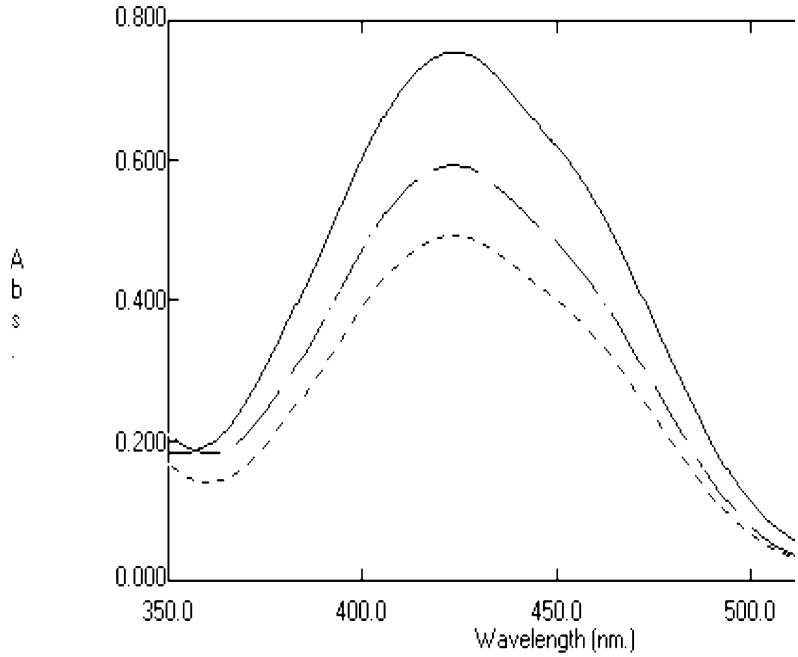
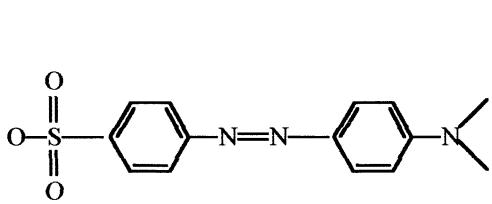
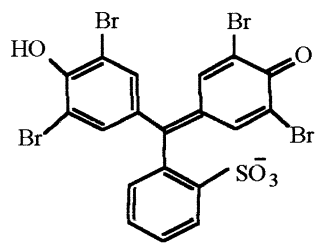


Fig. 2. Absorption curve of enrofloxacin (— —) and pefloxacin (— —): methyl orange ion pair complexes.



Methyl orange



Bromophenol blue

where at pH 2.3 or 2.5, only sulphonic acid group of BPB dissociates [15,16]. The yellow chloroformic extract with BPB showed maximum absorbance at 420 nm, while with MO at 424 nm for both enrofloxacin and pefloxacin (Figs. 1 and 2).

The pH of the aqueous phase is critical for colour formation, so the optimum pH was studied for each drug. In case of BPB, pH 2.4–2.6 and 2.2–2.4 were found to be the optimum for enrofloxacin and pefloxacin, respectively, while in case of MO, the optimum pH was 3.5–3.7 for both drugs.

Acetic acid sodium acetate buffer serves well in maintaining the proper pH in the range of the aforementioned pHs. Potassium chloride is included in these buffers merely as an aid in affecting complete separation of the organic phase and aqueous layer. The volume of buffer solution added were studied, complete colour development was attained by adding 4 ml buffer solution pH (2.5 and 2.3) for enrofloxacin and pefloxacin, respectively, for BPB method and pH 3.6 for both drugs with MO method.

The amount of BPB or MO should be sufficient enough and it was found that 5 ml of BPB or MO give the maximum absorbance and the excess has no effect on colour intensity. Addition of ethanol after extraction is necessary to prevent adsorption to the wall of the flask, and 1 ml was sufficient. The extracted ion pair was stable for 48 and 8 h

Table 1
Characteristic parameters for complexation of enrofloxacin and pefloxacin with BPB and MO

Parameters	Enrofloxacin		Pefloxacin	
	BPB method	MO method	BPB method	MO method
λ_{max} (nm)	420	424	420	424
Beer's law limits (μg ml^{-1})	2–12	1–12	2–18	4–40
Regression equation				
Slope (specific absorptivity)	0.7684	0.7719	0.4878	0.1964
R.S.D. (%)	0.85	1.21	1.32	0.95
Intercept	0.0593	0.0159	0.0089	0.0261
R.S.D. (%)	0.92	1.12	0.86	1.20
Correlation coefficient (r)	0.9953	0.9995	0.9934	0.9980

for enrofloxacin and pefloxacin, respectively, with BPB and for 48 h with MO for both drugs. No interference were observed in the determination of pefloxacin in the presence of the common excipients of the tablets, i.e. talc, magnesium stearate, starch, lactose, glucose and sucrose.

3.1. Method validation

Under the experimental conditions described, standard calibration curves for enrofloxacin and pefloxacin with BPB and MO were constructed by plotting absorbance, against concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 1. Also, the linear regression equation for each method is listed in Table 1. The correlation coefficients were 0.9934–0.9995 indicating good linearity.

The accuracy of the method was determined by investigating the recovery of enrofloxacin and pefloxacin at five levels ranging from 50 to 150% of the method concentration (0.1, 0.05 mg ml^{-1} enrofloxacin and pefloxacin, respectively, for BPB method and 0.1, 0.2 mg ml^{-1} enrofloxacin and pefloxacin, respectively, for MO method) from solution-spiked placebo.

Assays were performed in duplicate on two samples at five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for each drug determined by the proposed methods.

Spiked placebo assays were used to determine accuracy and precision of the proposed methods. The results are shown in Table 2, which indicate excellent recoveries ranging from 98.1 to 101.6% and 98.8 to 101.2% with a mean of 99.6 and 99.71% (R.S.D. = 1.1 and 0.88%, $N = 10$) for enrofloxacin and pefloxacin respectively, with BPB method. Table 3 indicates the recovery of enrofloxacin and pefloxacin using MO method, which range from 98.7 to 101.1 and 98.6 to 101.4 with a mean of 99.72 and 99.63% (R.S.D. = 0.87 and 0.86%, $N = 10$).

The measurement precision was determined by performing ten replicate measurements of the methods concentration. The R.S.D. was found to

Table 2
Accuracy of BPB method determined by recovery of enrofloxacin (**I**) and pefloxacin (**II**) from placebo tablets

Level %	μg added		Recovery %	
	I	**II**	**I**	**II**
50	50	50	100.5	99.2
50	55	52	101.6	100.3
75	75	75	100.8	101.2
75	76	76	99.3	99.1
100	100	100	99.0	98.9
100	107	102	99.9	100.1
125	125	125	98.7	101.0
125	120	126	98.1	99.0
150	150	150	99.0	98.8
150	158	152	99.0	99.5
Average			99.6	99.71
R.S.D. (%)			1.1	0.88

Table 3
Accuracy of MO method determined by recovery of enrofloxacin (**I**) and pefloxacin (**II**) from placebo tablets

Level %	μg added		Recovery %	
	I	**II**	**I**	**II**
50	25	100	99.1	100.3
50	25.6	101	100.2	101.4
75	37.5	150	101.0	100.5
75	38.1	149.5	99.3	99.3
100	50.0	200	98.9	99.1
100	51.0	200.3	100.3	99.5
125	62.5	250	101.1	98.8
125	61.4	251	99.2	99.2
150	75.0	300	99.4	98.6
150	76.1	300.6	98.7	99.6
Average			99.72	99.63
R.S.D. (%)			0.87	0.86

be 0.43 and 1.05% for enrofloxacin and pefloxacin, respectively, with BPB method and 0.86 and 1.35% for enrofloxacin and pefloxacin, respectively, with MO method (Table 4).

The results of accuracy and precision show that the proposed methods have good repeatability and reproducibility. Also the assay results are unaffected by the presence of excipients, this establish specificity of the methods. To ensure the validity of analytical procedure whenever used,

Table 4
Measurement precision of enrofloxacin (**I**) and pefloxacin (**II**) using BPB and MO method

Measurements	Absorbance			
	BPB method		MO method	
	I	**II**	**I**	**II**
1	0.827	0.478	0.756	0.223
2	0.826	0.472	0.750	0.227
3	0.824	0.470	0.760	0.230
4	0.822	0.480	0.751	0.223
5	0.829	0.475	0.762	0.225
6	0.830	0.482	0.752	0.231
7	0.821	0.473	0.759	0.229
8	0.825	0.471	0.756	0.226
9	0.820	0.485	0.763	0.231
10	0.821	0.477	0.755	0.224
Average	0.825	0.476	0.752	0.227
R.S.D. %	0.43	1.05	0.86	1.35

the stability of analytical solutions of enrofloxacin and pefloxacin during the analytical procedures were studied and the two analytes were stable for at least 24 h. To evaluate robustness and show reliability of the analytical procedure, different parameters affecting the procedures are studied. The proposed methods complied with USP [17] validation guidelines.

3.2. Tablet analysis

The proposed methods were applied for the determination of enrofloxacin and pefloxacin in Enroxil oral solution, Peflacine tablets and Peflacine ampoules. Five replicate determinations were made, satisfactory results were obtained for both drugs (Table 5).

The standard addition method was applied by adding pefloxacin to the previously analysed tablets, to check the validity of the proposed methods. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of the analysis of commercial tablets and the recovery study (standard addition method) of the drug, Table 5 suggested that there is no interference from any excipients, which may be present in tablets.

The results of determination of enrofloxacin and pefloxacin in Enroxil oral solution, Peflacine tablets and Peflacine ampoules obtained from BPB and MO methods were compared with reported methods [12,18], statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and F-ratio at 95% confidence level (Table 5), there is no significant difference.

4. Conclusion

Statistical comparison for the results of the proposed methods with reported methods indicate that there is no significant difference with regard to accuracy and precision. The principal advantage of the proposed methods is their suitability for the quality control of the drug alone and in

Table 5
Determination of enrofloxacin and pefloxacin in Enroxil oral solution, Peflacine tablets and Peflacine ampoules using BPB and MO methods compared with reported methods

Method	Values	Enroxil oral solution		Peflacine tablets		Peflacine ampoules	
		Proposed method	Reported method [12]	Proposed method	Reported method [18]	Proposed method [12]	Reported method [18]
BPB	Mean ± S.D.	99.5 ± 0.99	100.46 ± 0.87	99.39 ± 1.05	99.98 ± 0.78	100.02 ± 0.895	99.98 ± 0.78
	N	6	5	6	6	6	6
	t	2.005 (2.262)*		1.104 (2.228)*		0.082 (2.228)*	
	F	1.29 (6.26)*		1.57 (5.05)*		1.17 (5.05)*	
MO	Mean ± S.D.	100.3 ± 0.892	100.64 ± 0.87	100.25 ± 0.98	99.98 ± 0.78	100.2 ± 0.72	99.98 ± 0.78
	N	6	5	6	6	6	6
	t	0.637 (2.262)*		0.528 (2.228)*		0.5 (2.228)*	
	F	1.05 (6.26)*	1.57 (5.05)*		1.17 (5.05)*		

*Theoretical t and F values at $P = 0.05$.

tablets without fear of interference caused by the excipients expected to be present in tablets. The proposed methods compared with the charge transfer complex formation method and many already existing photometric methods are more sensitive, accurate and rapid.

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