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Quantitative determination of pefloxacin mesylate by residual-base neutralisation method

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Abstract: This work describes two procedures based on residual base determination for the quantification of pefloxacin mesylate (PFM) in bulk drug and in pharmaceutical products. In the first method involving titrimetry, the drug solution is treated with a measured excess of sodium hydroxide followed by back titration of the residual base with hydrochloric acid using a phenol red-bromothymol blue mixed indicator. The second spectrophotometrie method involves treatment of a fixed amount of sodium hydroxide – phenol red mixture with varying amounts of the drug, and measuring the decrease in the absorbance of the dye at 560 nm. In the titrimetric method, a reaction stoichiometry of 1:1 was found in the quantification range of 4–20 mg of drug. The spectrophotometric method allows the determination of PFM in the 5–40 μg ml^{-1} range. The molar absorptivity is 5.91×10^3 l mol^{-1} cm^{-1} and the Sandell sensitivity is 56.37 ng cm^{-2}. The methods were applied successfully to the determination of PFM in pharmaceutical preparations.

Keywords: pefloxacin, determination, titrimetry, spectrophotometry, pharmaceuticals.

INTRODUCTION

Pefloxacin (Fig. 1), [1-ethyl-6-fluro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid] is an antibacterial quinoline derivative.[1] It acts by inhibiting the bacterial enzyme DNA gyrase which is responsible for supercoiling of DNA. Many methods based on techniques such as fluorimetry,[2,3] TLC - fluorescence spectrodensitometry[4] and high performance liquid chromatography [HPLC][5–10] are available for the determination of the drug in body fluids. A number of analytical methods for the quantitative determination of PFM in pharmaceutical products are known. Procedures based on HPLC,[11,12] spectrophotometry,[13,14] capillary electrophoresis,[15] polarography,[16,17] voltammetry,[18] UV-spectrophotometry[19–22] and derivative spectrophotometry[23] are to be found in the literature. Though these methods[11–14,16,17] are sensitive, they require expensive instruments and trained personnel.

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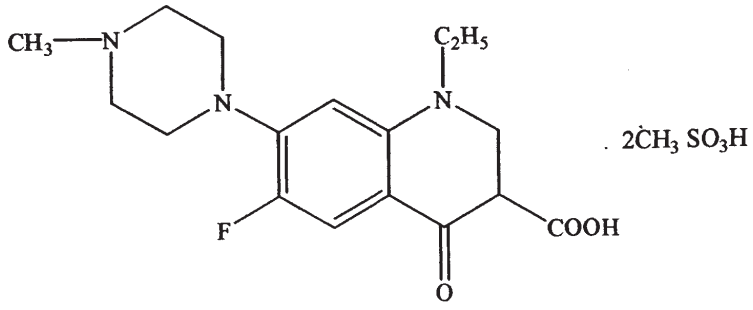

Fig. 1. Structure of PFM.

Despite the availability of sophisticated and sensitive instruments for the assay of pharmaceuticals, titrimetry and visible spectrophotometry continue to be the techniques of choice in laboratories where modern and expensive instruments are not available, because these techniques are simple, inexpensive, reproducible and accurate. A literature survey revealed that only one titrimetric method,[24] using sodium tetraphenuyl borate as the reagent, has been reported for the determination of PFM. However, the procedure in laborious and cumbersome since it inovles precipitation, filtration and back titration of the residual reagent with benzyldimethylalkylammonium bromide. Several visible spectrophotometric methods based on redox,[25] complexation,[26–28] ion – association complexation,[29,30] charge-transfer complexation[31] and oxidative coupling[32] reactions have been proposed. However, no work has been reported using a neutralization reaction for the determination of PFM. The present paper reports two methods based on a neutralization reaction.

EXPERIMENTAL

Apparatus

All absorbance measurements were made using a Systonics model 106 digital spectrophotometer provided with 1 cm matched quartz cells. An Equip tronics model EQ 614 digital pH meter was used for checking the pH values.

Reagents

All chemicals used were of analytical grade, and all solutions were freshly prepared in doubly distilled water (pH 7.0).

An approximately 0.01 M NaOH soltuion was prepared by dissolving about 0.4 g of the chemical (S.d.Fine Chem., India) in 1 liter of water followed by standardization with pure potassium hydrogen phthalate.[33] A 0.01 M HCl solution was prepared by diluting ≈ 0.90 ml of concentrated acid (Qualigens, India) Sp. Gr 1.18 to 1 liter with water. A phenol red-bromothymol blue mixed indicator was prepared by mixing equal volume of 0.1 % aqueous solutions of phenol red (Lobo Chem. India) and bromothymol blue (S.d. Fine Chem. India). A 0.8 mM NaOH–1.411 mM phenol red dye mixture was prepared in water of pH 7.

A stock standard solution of PFM, certified to contain 99.8 % active ingredient, equivalent to 2 mg ml^{-1}, was prepared by dissolving 200 mg of the pure drug in water and diluting to the mark in a 100 ml volumetric flask. For spectrophotometry, a working solution of 200 μg ml^{-1} was obtained by appropriate dilution of the stock solution.

Determination of PFM

Titrimetry. A 10 ml aliquot of standard sample solution containing 4 – 20 mg of PFM was accurately measured into a 100 ml titration flask, followed by the addition of 10 ml of 0.01 M NaOH by means of a pipette. The contents were mixed and the residual base was titrated with 0.01 M HCl using 3 drops of phenol red-bromothymol blue mixed indicator to the yellow colour end point. A 10 ml pure aqueous solution was used as the blank. The amount of drug was calculated from the amount of NaOH reacted.

Spectrophotometry. Different aliquots (0.25 – 2.0 ml) of 200 μg ml^{-1} PFM standard solution were delivered into a series of 10 ml volumetric flasks by means of a micro burette. An exactly measured 2 ml volume of alkali-dye mixture was added to each flask and the volume was made up to the mark with water, mixed well and the absorbance measured at 560 nm against a reagent blank. The absorbance values at 560 nm were plotted against the concentration of drug to obtain the calibration graph. The concentration of the unknown was read from the calibration graph or calculated from the regression equation computed from the Beer's law data.

Determination of PFM in tablets. Twenty tablets were weighed and ground into a fine powder. An amount of the powder equivalent to 200 mg of the active component was accurately weighed into a 100 ml volumetric flask, 60 ml of water added and the flask was shaken for 20 min. Then, the volume was diluted to the mark, mixed well and filtered using a Whatmann No. 42 filter paper. The first 10 ml portion of the filtrate was discarded, and a suitable aliquot of the filtrate was subjected to analysis by titrimetry. An adequate volume of the extract (2 mg ml^{-1}) was appropriately diluted to obtain a 200 μg ml^{-1} solution which was then analysed by spectrophotometry by taking convenient volumes as described under the determination of PFM.

Determination of PFM in injection solutions. The injection solution equivalent of 200 mg of PFM was diluted with water to 100 ml a volumetric flask and a suitable aliquot was analysed by titrimetry. The stock solution was diluted to 200 μg ml^{-1} and analysed by spectrophotometry.

RESULTS AND DISCUSSION

Both methods are indirect and are based on the determination of the residual base after treating the drug with a measured excess of base.

Titrimetry. PFM is a weak acid and direct titration with a strong base in aqueous medium leads to erroneous results owing to the difficulty of locating the end point with visual indicators. For non-aqueous titration, the medium should be scrupulously anydrous and even traces of water yield erroneous results. In the proposed method, PFM is treated with a known excess of NaOH and the residual base is back titrated with HCl. Of the various mixed indicatorst tried, phenol red-bromothymol blue gave a sharp end-point. Using 0.01 M NaOH and HCl, 4 – 20 mg of PFM can be conveniently determined. The reaction stioichiometry was found to be 1 : 1 which served as the basis for the calculations. The relation between the amount of drug and the titration end point was evaluated by calculating the regression coefficient, *r*, *via* the regression equation.

The value was found to be – 0.9986 revealing that the reaction between the drug and NaOH proceeds stoichiometrically (1:1) in the investigated range (4–20 mg).

Spectrophotometry. The method using phenol red is based on the fact that the colour of the dye is controlled by the pH of the solution and that the colour change is not abrupt but occurs in a continuous manner when the pH changes continuously. Phenol red changes its colour from red to yellow over a pH change from 8.2 to 6.8 (transition inter-

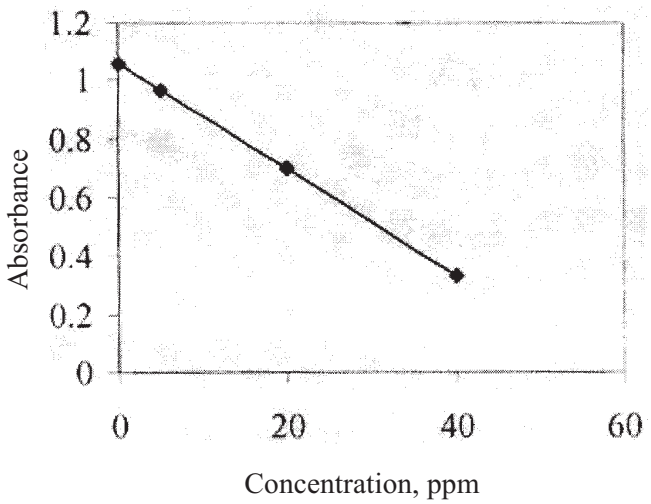

Fig. 2. Beer's law dependence.

val). When PFM is added in increasing amounts to a fixed amount of base – dye mixture, the pH is progressively reduced because of the neutralization of the base by the drug, and, as a result, the intensity of the red colour of the solution decreases. This is indicated by the proportional decrease in the absorbance of the solution at 560 nm, which is corroborated by the correlation coefficient, $r = -0.9992$ (Fig. 2). This decreases in absorbance continues till a pH of 6.8 is reached when the dye changes to its yellow form.

TABLE I. Accuracy and precision

Titrimetry				Spectrophotometry			
Amount of drug taken mg	Amount of drug found* mg	Relative error#/%	RSD/%	Amount of drug taken μg	Amount of drug found* μg	Relative error/%	RSD/%
8.00	8.12	1.50	2.47	100.00	101.03	1.03	2.38
12.00	12.20	1.67	0.82	200.00	198.21	0.89	1.92
16.00	15.81	1.19	0.95	300.00	303.39	1.13	2.63

*Mean values of seven determinations; % error = $\frac{|\text{Observed value} - \text{True value}|}{\text{True value}} \times 100$

In a preliminary study, a 10 μg ml^{-1} solution of phenol red in 0.5 NaOH was found to produce an absorbance of 1.01 at 560 nm. A 2 ml volume of 0.8 mM NaOH–50 μg ml^{-1} phenol red dye mixture in a total volume of 10 ml produced the same absorbance. When different amounts of drug were added to this fixed amount of base – dye mixture (2 ml of 0.8 mM NaOH – 50 μg ml^{-1} dye), the absorbance decreased due to the neutralisation of the NaOH and reached a constant value for a drug concentration of 40 μg ml^{-1}, thus enabling the lower and upper limits of the linear range of applicability to be fixed.

TABLE II. Results of the analysis of pharmaceutical formulations containing PFM

Formulation*	Label claim mg/tab or mg/50 ml	% Recovery ± *SD*			Student's *t*-value+		*F*-value++	
		Titrimetry (T)	Spectrophotometry (S)	Reference method[34]	T	S	T	S
Peflox tablet[a]	200	102.15 ± 1.11	101.05 ± 1.72	100.90 ± 1.19	1.72	0.16	0.71	2.09
	400	98.65 ± 1.21	99.25 ± 1.33	98.40 ± 1.12	0.34	1.09	0.18	1.41
Pebid tablet[b]	400	100.41 ± 1.45	102.32 ± 1.51	100.91 ± 1.32	0.57	1.58	1.21	1.31
Qucin tablet[c]	400	99.10 ± 1.45	99.81 ± 1.22	100.92 ± 1.23	2.15	1.43	1.39	1.02
Peflobid injection	100	100.98 ± 1.12	99.13 ± 1.32	101.11 ± 1.44	0.16	2.27	1.65	1.19

*Marketed by a: Woekhardt; b: Indehemie; c: Aristo, India; + Tabulated value at 95 % confidence level is 2.77; ++ Tabulated value at 95 % confidence level is 6.39

TABLE III. Results of recovey study by the standard – addition method

Formulations*	Titrimetry				Spectrophotometry			
	Amount of PFM in formulation/mg	Amount of PFM standard added/mg	Total found mg	% Recovery of PFM standard added*	Amount of PFM in formulation/μg	Amount of PFM standard added/μg	Total found μg	% Recovery opf PFM standard added*
Peflox tablet	5.11	5.00	10.29	103.60	50.53	100.00	150.93	100.40
(200 mg)	5.11	9.00	13.91	97.78	50.53	200.00	251.21	100.34
	5.11	12.00	17.3	101.83	50.53	300.00	354.41	101.29
Pebid tablet	5.02	5.00	10.00	99.60	51.16	100.00	150.33	99.17
(400 mg)	5.02	9.00	14.33	103.44	51.16	200.00	254.41	101.63
	5.02	12.00	17.21	101.58	51.16	300.00	348.81	99.22

* Mean values of three determinations

Beer's law was obeyed over a drug concentration range of 5 – 40 μg ml^{-1} (Fig. 2). The apparent molar absorptivity was 5.91 × 10^3 l mol^{-1} cm^{-1} and the Sandell sensitivity was 56.37 ng cm^{-2}. The linear plot gave the regression equation: A = 1.0528 – 0.0179 c, where A is the absorbance and c the along concentration in μg ml^{-1}. The correlation coefficient was 0.9992 (n = 8). The limit of deterction was 6.48 μg ml^{-1} and the limit of determination 21.59 μg ml^{-1}.

Accuracy and precision of the methods. Under the optimum conditions, the accuracy and precision of the proposed methods were determined by performing seven replicate analyses of pure drug solution at three levels (amounts). The results of the study are presented in Table I and are indicative of the good accuracy and precision of the methods.

Application. The methods were applied to the determination of formulations of tablets and injection solutions containing PFM. The results are presented in Table II. The validity of the methods was tested by analysing the same batch preparations by the official method[34] which involves non-aqueous titration with acetous percholoric acid for the pure drug and UV-spectrophotometry for formulations. Statistical analysis of the results revealed that at the 95 % confidence level, the calculated t- and F-values are less than the reference values (Table II) indicating that the proposed methods and the official method are comparable in terms of accuracy and precision.

The accuracy and reliability of the methods were further ascertained through recovery studies. To a fixed and known amount of drug in the pre-analysed formulation, pure PFM was added at three different levels and the total was found by the proposed methos. The percent recoveries of the added pure drug presented in Table III reveal that neither the end-point detection in titrimetry nor the absorbance measurement in spectrophotometry was affected by the commonly encountered excipients and diluents, such as talc, starch, sodium alginate, magnesium stearate, lactose, calcium gluconate and calcium hydrogen phosphate.

CONCLUSIONS

The methods described in this paper, which are based on the determination of the residual base, are novel, simple, relatively specific, accurate and precise for the determination of PFM. The titrimetric method is straightforward and fast when compared to the time – consuming precipitation method reported earlier.[24] The spectrophotometric method employs mild working conditions without heating or extraction[29] and is as sensitive as many reported methods. The method is highly reliable owing to the stability of the dye colour which is ultimately measured which is amply demonstrated by the high reproducibility of the results. These merits, besides the use of simple and inexpensive reagents and instruments, recommend the use of the methods in drug control laboratories.

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ИЗВОД

КВАНТИАТИВНО ОДРЕЂИВАЊЕ ПЕФЛОКСАЦИН МЕСИЛАТА МЕТОДОМ НЕУТРАЛИЗАЦИЈЕ ЗАОСТАЛЕ БАЗЕ

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У овом раду описана су два поступка заснована на одређивању заостале базе, за квантитативно одређивање пефлоксацин месилата (PFM) у лековима и фармацеутским производима. Прва метода заснована је на титриметрији, код које се раствор лека третира одмереним вишком натријум-хидроксида и у којем се повратно титрише неутрошена база хлороводоничном киселином уз коришћење фенол црвено – бромтимол плаво мешовитим индикатором. Друга је спектрофотометријска метода у којој се фиксираним количинама смеше натријум-хидроксида и фенол црвеног додају различите количине лека и мери смањење апсорбанције боје на 560 nm. Код титриметријске методе је нађена реакциона стехиометрија 1:1 у опсегу количина лека од 4 – 20 mg. Спектрофотометријска метода омогућава одређивање у опсегу концентрација 5 – 40 μg ml^{-1}. Моларна апсорптивност је $5{,}91\times10^3$ l mol^{-1} cm^{-1} а Sandell-ова осетљивост 56,37 ng cm^{-2}. Метода је успешно примењена за одређивање PFM у фармацеутским препаратима.

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