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Spectrophotometric Estimation of Fluroquinolones as Ion-Pairs with Bromocresol Green in Bulk and Pharmaceutical Dosage Form

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A simple, rapid and sensitive spectrophotometric method has been proposed for the estimation of fluroquinolones *viz*., norfloxacin (NRF), ciprofloxacin (CPF), ofloxacin (OFL) and moxifloxacin (MXL) in bulk and pharmaceutical formulations. The method is based on the reaction of selected drugs with bromocresol green (BCG) in neutral medium for NRF, CPF and OFL and in acetate buffer of pH 3.7 for MXL to give chloroform soluble ion association complexes. The effects of various parameters have been studied. The ion association complexes exhibited absorption maxima at 421.4 nm for NRF, 418.8 nm for CPF, 417.4 nm for OFL and 415.8 nm for MXF. Beer-Lambert's law were obeyed in the concentration ranges of 2-24, 2-10, 2-12 and 2-20 µg/mL for NRF, CPF, OFL and MXL, respectively with correlation coefficient not less than 0.9998. The results obtained by the proposed methods have been statistically compared by means of student t- test and by the variance ratio F-test.

Key Words: Fluroquinolones, Bromocresol green, Ion-pair extraction, Spectrophotometry.

INTRODUCTION

Quinolones comprise an interesting group of antibacterial, whose action is based on their anti-DNA activity. They all possess a carboxylic acid group at position 3 and carbonyl group in position 4. Hence they are referred as 4-quinolones. Their antibacterial activity is remarkably increased by the addition of 6-fluro and 7-piperazinyl group to the molecule and named fluroquinolone. They are the second-generation members of antibacterial quinolones and are greatly effective against both gram negative and gram positive pathogens that are resistant to the other antibacterials[1].

Several methods have been reported for the determination of quinolones in pure form, in dosage forms and in biological fluids. Norfloxacin (NRF) and ciprofloxacin (CPF) are official in USP[2], BP[3] and IP[4], while ofloxacin (OFL) is official in USP[5] only. Both USP and BP recommend HPLC method for the determination of CPF in raw materials and in dosage form. The USP recommends non-aqueous titration methods for determination of NRF and OFL in raw materials

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while HPLC methods are described for analysis of their dosage forms. The BP recommends a non-aqueous titration method for determination of norfloxacin in raw material and spectrophotometric method for determination of norfloxacin in dosage forms. Several methods for the determination of quinolones including titrimetric[6], spectrophotometric[7-13], spectroflurometric[7] and chromatographic[14,15] are available in literature. Most of the analytical methods employed for the determination of the studied drugs in biological fluids are HPLC methods[16-23], which require complex and expensive equipment, provision for use and disposal of solvents, sample preparation procedure and personnel skilled in chromatographic techniques.

The present study is based on the reaction of selected drugs with bromocresol green (BCG) in neutral medium for NRF, CPF and OFL and in acetate buffer of pH 3.7 for MXL to give chloroform soluble ion association complexes. The proposed method is simple, sensitive, accurate and readily applied to the analysis of selected drugs in pharmaceutical formulations.

EXPERIMENTAL

All absorbance measurements were made on a Shimadzu UV 1601 UV-visible double beam spectrophotometer with 1 cm matched quartz cells.

All chemicals used were of analytical grade. Pure NRF and CPF were obtained from Makers Laboratories Ltd., Mumbai, OFL and MXL were obtained from Alkem Lab. Ltd., Raigad and Cipla Ltd., Mumbai, respectively as a gift samples.

The solutions of standard NRF, CPF, OFL and MXL (1mg/mL) were prepared in N/2 acetic acid. Working standard solution of NRF, CPF, OFL and MXL (100 µg/mL) were prepared by diluting standard solutions with N/2 acetic acid. Bromocresol green (BCG) solution was prepared by dissolving 50 mg of bromocresol green in 1.5 mL of ethanol and diluting with ethanol to 100 mL in calibrated volumetric flask. Acetate buffer solution (pH 3.7) was prepared as per IP 96.

Assay procedure: Aliquots of working standard solution containing 0.02-0.25, 0.02-0.10, 0.02-0.10 and 0.02-0.20 mg of NRF, CPF, OFL and MXL, respectively, were transferred into a series of 25 mL separating funnel, 1 mL BCG solution and 4.0 mL of acetate buffer solution in case of MXL were added in each funnel. The reaction mixture was extracted with 10 mL chloroform by shaking for 1 min at vortex. The yellow coloured organic layers were taken and the absorbance at 421.4 nm for NRF, 418.8 nm for CPF, 417.4 nm for OFL and 415.8 nm for MXF against the reagent blank. A calibration graph of the absorbance *vs.* the concentration of the selected drugs was plotted.

Assay procedure for pharmaceutical formulation: Twenty tablets of the selected drugs were finely powdered. An amount equivalent to 50 mg of the drug was weighed accurately and transferred into 100 mL beaker. Using a mechanical stirrer the powder was completely disintegrated in N/2 acetic acid filtered through a Whatman filter paper No. 40 and diluted up to 50 mL with N/2 acetic acid. It was mixed well and the same procedure applied as described under the procedure for the pure samples.

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 are given in literature[10,11]. Norfloxacin (NRF), ciprofloxacin (CPF) and ofloxacin (OFL) are amino compounds containing piperazine ring. Ion-pairs formed between these drugs and bromocresol green can be used for its determination in bulk and pharmaceutical formulation. In this study, extractive spectrophtometric method was developed based on the reaction of selected drugs with bromocresol green (BCG) in neutral medium for NRF, CPF and OFL and in acetate buffer of pH 3.7 for MXL to give chloroform soluble ion association complexes. Then the linearity, accuracy, precision, sensitivity, stability of the proposed method was described and applied to pharmaceutical formulation as tablet and the obtained results evaluated statistically.

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

Selection of the wavelengths: The absorption spectra of the ion pair complexes, formed between selected drugs and BCG were measured at 360-550 nm against the reagent blank. The yellow coloured extracts shows maximum absorbance at 421.4 nm for NRF, 418.8 nm for CPF, 417.4 nm for OFL and 415.8 nm for MXF. The measurements were made at these wavelengths for pure sample and pharmaceutical formulations.

Selection of the extracting solvents: The effect of the extracting solvents on the ion pair complexes was examined. Chloroform was found to be the most suitable for the quantitative extraction of the complex because of its slightly higher efficiency on colour intensity, selective extraction of the drug-dye complex from the aqueous phase and obtained highest absorbance with chloroform.

Effect of pH buffer: The effect of a buffer in the formation of ion association complexes was studied by extracting a coloured complex in the presence of various buffers such as potassium hydrogen phthalate buffer (pH 2.2-4.0) and acetate buffer (pH 2.8-4.0). It was noticed that the maximum colour intensity and constant absorbance were found in acetate buffer of pH 3.7 for MXL-BCG system. However, the NRF-BCG, CPF-BCG and OFL-BCG systems gave constant and maximum absorbance in only a neutral medium.

Dye concentration: The effect of the dye concentration on the intensity of the colour developed at selected wavelength was studied using different mL of the reagent. The result shows that 1 mL of BCG was found to be optimum for this proposed method and excess of dye do not affect the colour of the complex or the absorbance.

Stoichiometric relationship: The composition of complexes was determined by Job's method of continuous variation and the results correspond to 1:1 for the drug to dye ratio.

Stability of ion-pair complexes: The stability of the ion pair complexes between selected drugs and dye (BCG) was evaluated. The absorbances of the complexes were found to be stable for more than 12 h.

Method validation

Linearity of the calibration curves: The Beer's law limits and the molar absorptivity values were evaluated. The regression analysis indicated linear relationship between absorbance and concentration. The graphs of the absorbance *vs.* the concentration showed zero intercepts and are described by the regression equation Y = a + bx. The results are summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA

	NRF	CPF	OFL	MXL
λ_{max} (nm)	421.4	418.8	417.4	415.8
Beer's law limits (µg/mL)	2-24	2-10	2-12	2-20
Molar absorptivity (L mol^{-1} cm^{-1})	3959	10768	14456	11116
Correlation coefficient (r)	0.9941	0.9999	0.9999	0.9999
Slope, b	0.0120	0.0331	0.0402	0.0256
Intercept, a	0.0629	0.0664	0.0173	0.0116
% Range of error (at 95 % confidence limit)	0.1838	0.2619	0.2387	0.1909

Specificity, precision, accuracy and ruggedness: Specificity of ion-pair reaction, selective determination of selected drugs, the basic nitrogenous compounds with BCG could be possible. The accuracy of the method was established by analyzing the pure drugs at five concentration levels and the precision was carried out by determining the relative standard deviation (RSD) of six determinations at five different concentration of each drug. The low values of RSD and range of error at the 95 % confidence level indicate the high accuracy and precision of the method. In order to ascertain the ruggedness of the method, four replicate determination at different concentration levels of the drugs were carried out. The within-day RSD values were found to be less than 0.96 % for all drug solutions taken within the Beer's ranges, hence indicating that the proposed method has reasonable ruggedness.

Analysis of pharmaceutical preparations: The proposed method was applied to the estimation of NRF, CPF, OFL and MXL in pharmaceutical preparations. The recoveries calculated as:

$$\text{Recovery (\%)} = \text{measured content of drug/added drug} \times 100$$

The results of the proposed methods for tablets are summarized in Table-2. The preanalyzed tablet solutions were tested for possible interference in the standard addition method. The regression equations of standard addition curves were found for selected drugs by proposed method. There is no significant difference between slopes of proposed method with calibration curves and standard addition methods. Therefore it indicates that there is no spectral interaction in the analysis that the excipients in tablet forms of NRF, CPF, OFL and MXL.

TABLE-2
RESULT OF ANALYSIS OF SELECTED DRUGS IN PHARMACEUTICAL FORMULATIONS BY THE PROPOSED METHOD AND THEIR COMPARISON WITH OFFICIAL METHODS

Drug	Label claim (mg per tablet)	Recovery[a] % ± SD, % and their comparison with official method	
		Reported/official	Proposed
Norfloxacin[12]			
Tablet 1	200	99.37 ± 0.4660	99.57 ± 0.1744; F = 1.13; t = 0.85
Tablet 2	400	99.72 ± 0.4314	99.20 ± 0.5082; F = 1.38; t = 1.73
Ciprofloxacin[12]			
Tablet 1	250	99.30 ± 0.4662	99.60 ± 0.2087; F = 1.49; t = 1.33
Tablet 2	500	99.69 ± 0.2383	99.77 ± 0.2051; F = 1.34; t = 0.86
Ofloxacin[7]			
Tablet 1	200	99.82 ± 0.09783	99.69 ± 0.4074; F = 1.40; t = 0.73
Tablet 2	400	99.41 ± 0.4451	99.60 ± 0.4187; F = 1.50; t = 0.83
Moxifloxacin[13]			
Tablet 1	400	99.87 ± 0.0795	99.41 ± 0.5677; F = 1.37; t = 1.79
Tablet 2	400	99.71 ± 0.1686	99.81 ± 0.1348; F = 1.56; t = 1.05

a: Average of five determinations.

Comparison of the methods: The results of the analyses of pharmaceutical formulations were compared statistically by the t-test and by variance ratio F-test with those obtained by official/reported methods. The student t-values at the 95 % confidence level did not exceed the theoretical values, indicating there is no significance difference between the proposed and official/reported methods.

Conclusion

The proposed method is simple and rapid. The maximum colour development of drug-BCG ion pair complex formation is completed immediately after all reagents were added. All the measurements were made in 0.5 h after the preparation of the solution in all the experiments. This method does not involve procedural steps, take more operator time and expertise like HPLC and other methods. The validity of the proposed method is well demonstrated by analyzing tablet dosage forms of NRF, CPF, OFL and MXL. Moreover, the method is free from interferences by common additives and excipients. Thus the proposed method can be employed for the routine determination of fluroquinolones *viz*., norfloxacin, ciprofloxacin, ofloxacin and moxifloxacin in bulk and pharmaceutical formulations.

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