Original Research Article

Spectrophotometric ~~approach for the~~ determination of Ciprofloxacin hydrochloride ~~(CPFX)~~, Moxifloxacin hydrochloride ~~(MOXI)~~, and Roxithromycin hydrochloride ~~(ROXI)~~ in pure form

Abstract

A straightforward, sensitive, and quick spectrophotometric approach was created and validated for the detection of Ciprofloxacin hydrochloride (CPFX), Moxifloxacin hydrochloride (MOXI), and Roxithromycin hydrochloride (ROXI) in pure form. These techniques were based upon the fact that these antibiotics produced dark yellow ion pairs when combined with bromophenol blue (BPB). The binary complex in universal buffer solution of the optimum pH values was demonstrated at absorption maxima at 633 nm, 632 nm, and 633 nm for the three drugs, respectively, with bromophenol blue (BPB). Various parameters, such as the effect of time and the effect of reagent concentration, were optimized. Beer's law plots were obeyed in the concentration ranges 2– 8 µg ml^{-1}, 1– 4 µg ml^{-1}, and 1– 6 µg ml^{-1}, for the three drugs, respectively, with bromophenol blue (BPB)

Keywords: Ciprofloxacin hydrochloride; Moxifloxacin hydrochloride and Roxithromycin hydrochloride; Bromophenol blue; Ion pair complex.

NO NEED FOR ABBREVIATIONS IN THE ABSTRACT!

Introduction

Ciprofloxacin (CPFX) belongs to the fluoroquinolones. The

fluoroquinolones are a set of antibacterial agents that have been used science 1980 as a routine treatment for skin, gastrointestinal, respiratory, urinary, bone and joint infection. They are also used for treatment of sexually transmitted diseases. Ciprofloxacin is characterized by a high and rapid bactericidal activity. It acts through the suppression of the bacterial topoisomerase/DNA gyrase enzyme which in turn leads to inhibition of DNA synthesis in the bacterial cells. Fluoroquinolones have been associated with adverse side effects in patients, including gastrointestinal (vomiting, nausea, abdominal discomfort, and diarrhoea), CNS (insomnia, headache, depression, and tremors), and skin side effects.

After stopping the medicine, these side effects can be reversed and are not dose-dependent [1–5]. A fourth-generation synthetic 8-methoxyquinolone derivative of fluoroquinolone antibacterial drugs is moxifloxacin. It was found in 1999 by the insertion of an azabicyclo-substitution at C-7, which is connected to activity against both Gram-negative and Gram-positive bacteria as well as a wide range of pathogens. A methoxy group at the C-8 position has also been linked to improved efficacy against tuberculosis and a lower tendency to acquire phototoxicity resistance [6–9].

The semi-synthetic macrolide antibiotic roxithromycin is produced from erythromycin, which has long been used to treat infections in both humans and animals. However, roxithromycin is easily discharged into the environment through the excretions of humans and animals due to its lengthy elimination half-life in vivo. In recent years, roxithromycin, an emerging pollutant, has been frequently found in aquatic environments, including surface water, ground

water, and wastewater [10–14].

Acid phthalein dye bromophenolblue (BPB) is a blue colour. It serves as a pH indicator and is categorised as a Bronsted acidic or basic dye with a transition range of pH 3 to 4.6. (proton acidity and hydrogen bonding). The literature advises using bromophenol blue, which displays colours of yellow at pH 3.0 and blue at pH 4.6, for the titration of strong alkalis with strong acids. BPB is employed as a sensor to measure a variety of substances, including ammonia, medications, proteins, and amino acids [15–23].

Azo dyes have a high-water solubility and limited biodegradability, which negatively affects aquatic life by lowering dissolved oxygen levels and reducing light influx [24–27]. Several methods have been done in literature for the analysis of Ciprofloxacin hydrochloride, Moxifloxacin hydrochloride and Roxithromycin such as Colorimetric Method, Spectrophotometric method, Voltametric methods, Potentiometric determination HPLC, Capillary zone electrophoresis method, etc.

The research method relies on the development of colored (charge transfer or ion-pair) complexes between the drug and reagent that may be detected by a visual spectrophotometer. In an ion-pair complex, ions with opposing electric charges are attracted to one another in solution to create a unique chemical compound. It acts as one cohesive entity. Physical chemistry first looked into ion pair production, but chemical analysis, notably pharmaceutical analysis, has found it to be quite intriguing.

Experimental

Apparatus

All the absorption measurements are carried out using a Jasco UV/Vis spectrophotometer (model V-670, Jasco, Tokio, Japan) with scanning speed 400 nm/min, band width 2.0 nm, and equipped with 1.0 cm pair-matched quartz cells. The pH of all solutions was adjusted using a pH-meter (HI 8014, HANNA Instruments, Woonsocket, RI, USA). Spectrofluorimetric measurements were carried out using a spectrofluorometer (Fluoromax-4, Horbiba Scientific, Kyoto, Japan), with the slit widths for both excitation and emission set at 9 nm.

Materials and reagents

All materials used were of Analytical Reagent grade, doubly distilled water, and methanol were used throughout the work.

Preparation of solutions

Stock solutions of Ciprofloxacin hydrochloride, Roxithromycin and bromophenol were prepared in methanol while, Moxifloxacin hydrochloride solution was prepared in dist. water. The solutions were further diluted as per requirement.

Procedure for calibration curve

Suitable aliquots of ciprofloxacin hydrochloride or roxithromycin solutions in methanol or moxifloxacin hydrochloride in dist. water were transferred into 10 ml volumetric flasks. To it, 1 ml of $2x10^{-3}$ M bromophenol blue solution for ciprofloxacin hydrochloride, moxifloxacin hydrochloride and roxithromycin hydrochloride were added and volume was made up to 10 ml with respective solvents. This made the final concentration of bromophenol blue to 0.2 mM. the absorbance of dark yellow solution was measured at 633 nm, 632 nm and 633 nm against the appropriate reagent blank for bromophenol blue.

Procedure for dosage form

For analysis of tablets, five tablets were weighed and average weight of one tablet was determined. The contents of 5 tablets were weighed, ground into a fine powder and mixed. An accurately weighed portion of the powder equivalent to one tablet was transferred into a 50 mL volumetric flask. The volume was made up to the mark with water. After 30 min of mechanically shaking, the solution was filtrated in a 50 mL calibrated flask through Whatman No. 42 filter paper. Necessary amounts of filtrate were diluted to a 50 mL bidistilled water and the same procedure were applied as described under the procedure for bulk samples.

Results and Discussion

Effect of solvent

Various solvents like methanol, ethanol, acetone, dimethylsulphoxide, chloroform and acetonitrile were used to check the solubility, complex formation, to achieve maximum sensitivity and product stability. methanol for ciprofloxacin hydrochloride, roxithromycin and bromophenol blue and dist water for moxifloxacin hydrochloride and methyl orange were found to be most suitable solvents.

Absorption spectra

Solutions of ciprofloxacin hydrochloride or roxithromycin and

bromophenol blue in methanol and moxifloxacin hydrochloride and methyl orange in dist waer were prepared. Absorption spectra of these solutions were recorded individually. When the drug solutions were mixed with BPB solution, dark yellow complexes were formed with absorption maxima at 633 nm for ciprofloxacin hydrochloride, 632 nm for moxifloxacin hydrochloride and 633 nm for roxithromycin respectively. Under experimental conditions, the reagent as well as the drug showed negligible absorbance while the complexes showed maximum absorbance at these wavelengths. Hence, it was concluded that the studies for quantitative analysis could be carried out at these wavelengths.

Effect of pH

After a universal buffer solution was prepared different values of pH from 2.0 to 12.0 was added to fixed of studied drugs test and blank to select optimum pH(Figure 1). The optimum pH values were 5.12, 8.7 and 7.2 for CPFX , MOXI and ROXI (100 ppm) with BPB, respectively.

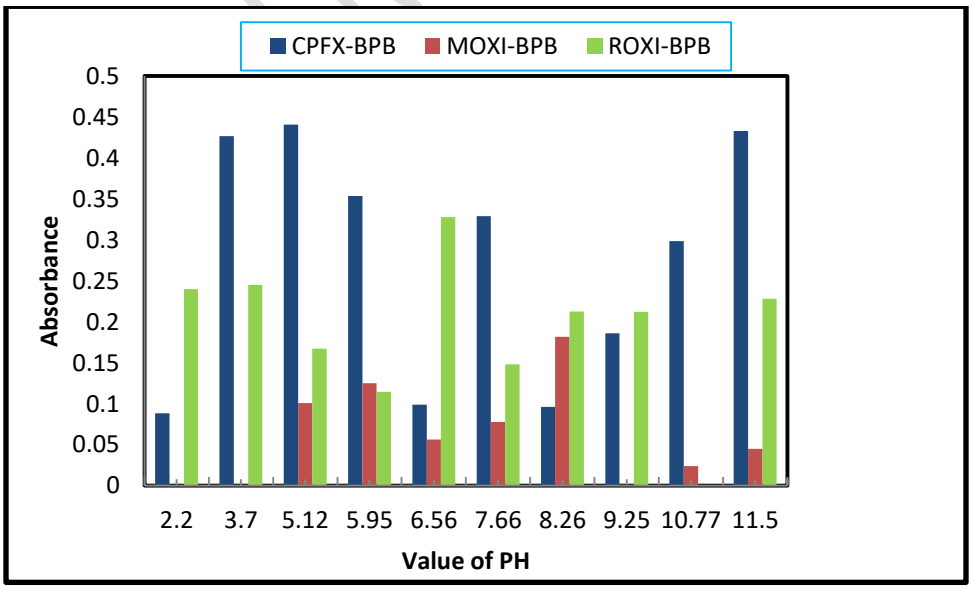

Figure 1: Effect of value of PH on ion pair complexes of CPFX , MOXI and ROXI (100 ppm) with BPB

Effect of time

Mixtures of drug and reagent were prepared; the optimum reaction time was determined by recording the absorbance of the formed complexes at different time intervals. The variation has been shown in Figure 2. After studying the effect of time on the complexes under consideration as shown (Fig. 2), it was found that CPFX, MOXI and ROXI complexes with BPB were formed instantaneously having maximum absorbance.

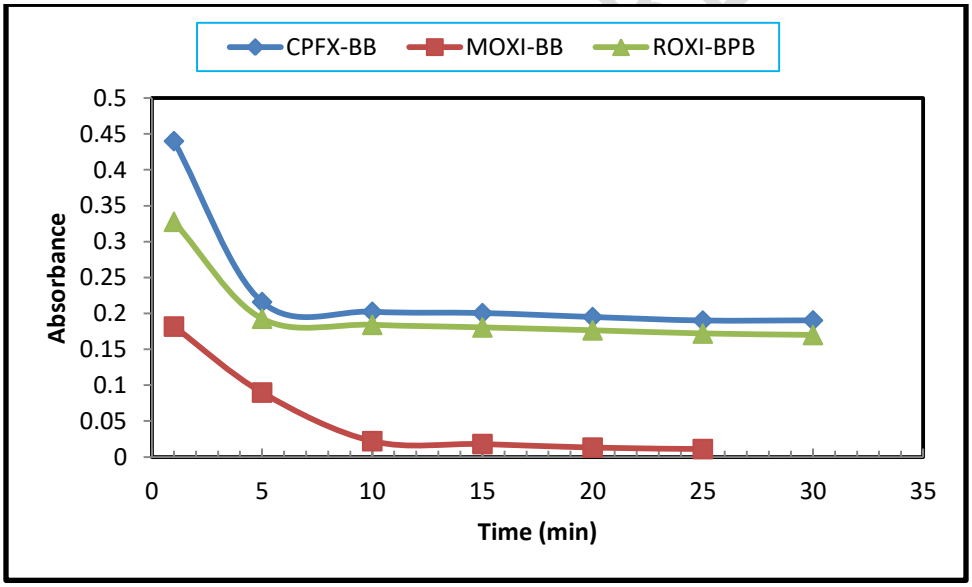

Figure 2: Effect of time on the absorbance of ion pair complexes of CPFX, MOXI and ROXI with BPB.

Effect of reagent concentration

The optimum concentration of bromophenol blue was determined by adding various volumes (0.5- 5 ml) of 2 mM bromophenol blue to the drugs. The color intensity and the absorbance were found to be maximum for ciprofloxacin hydrochloride, moxifloxacin hydrochloride and roxithromycin bu using 1 ml of 2mM BPB. Therefore, these concentrations were used to prepare calibration curve (Figure 3). All the observations were made in triplicate and mean of the three values have been plotted in each graph.

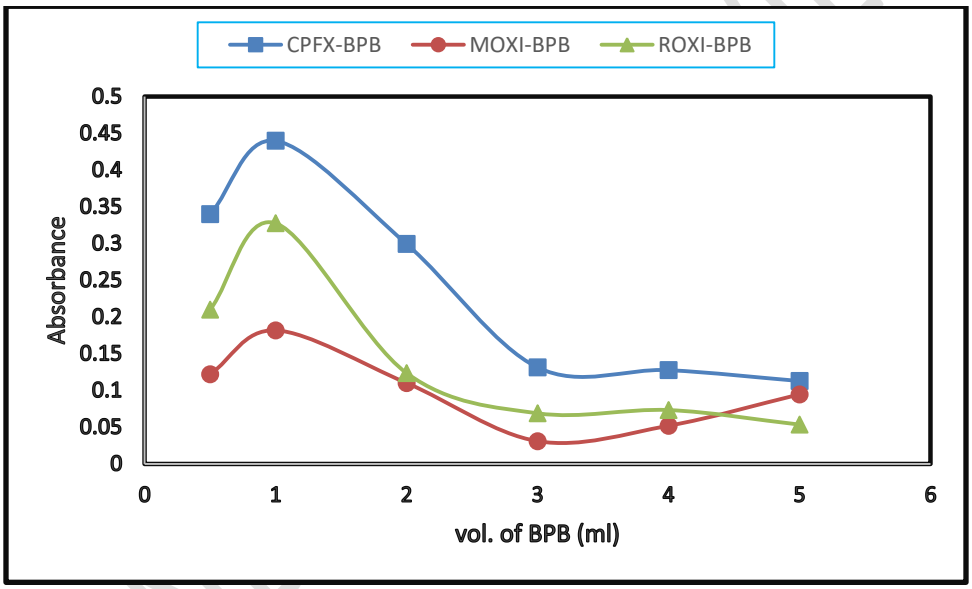

Figure 3: Effect of BPB concentrations on ion pair complexes of CPFX , MOXI and ROXI (100 ppm)

PLEASE EXPLAIN ABBREVIATIONS IN FIGURES!!!

Effect of sequence of mixing

The results of different sequences of addition to select the most suitable one for developing the concerned complexes is investigated by measuring the absorbance of solutions prepared by different sequences of addition in the visible region against a blank solution prepared in the same manner. Experiments showed that the order "reagent-drug-buffer" gave the best results for all complexes. Other sequences gave lower absorbance values under the same conditions (Figure 4).

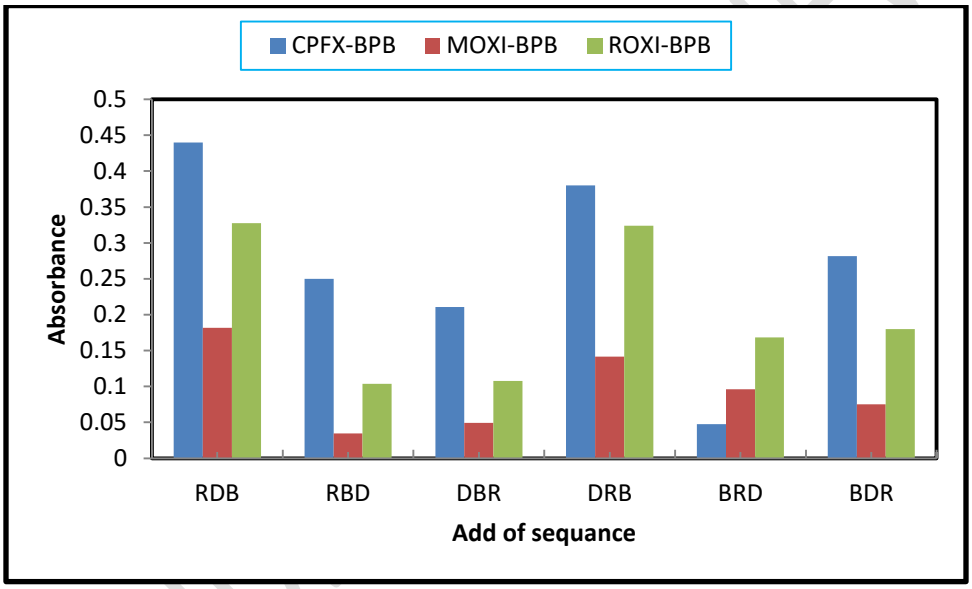

Figure 4: Effect of sequence of addition on ion pair complexes of CPFX , MOXI and ROXI (100 ppm) with BPB.

Stoichiometric relationship and stability studies

Composition and stability constants of these complexes were established by applying Job's method of continuous variation. Equimolar solutions of the

drug and the reagent were mixed in various proportions and absorbance of each mixture was recorded at optimum conditions. The results indicated that the complexes are formed in the ratio of 1:1 (D:R) for BPB (Figure 5). Mechanism of formation of such complexes with composition $(DH)^{+2}$ $(R)^{2-}$ has been discussed by Gainza and Konyeaso [28]. The stability constants (log K) values were found to be 6.34 ± 0.03 for ciprofloxacin hydrochloride, 5.22 ± 0.04 for moxifloxacin hydrochloride and 4.53 ± 0.06 for roxithromycin respectively showing high stability of the complexes.

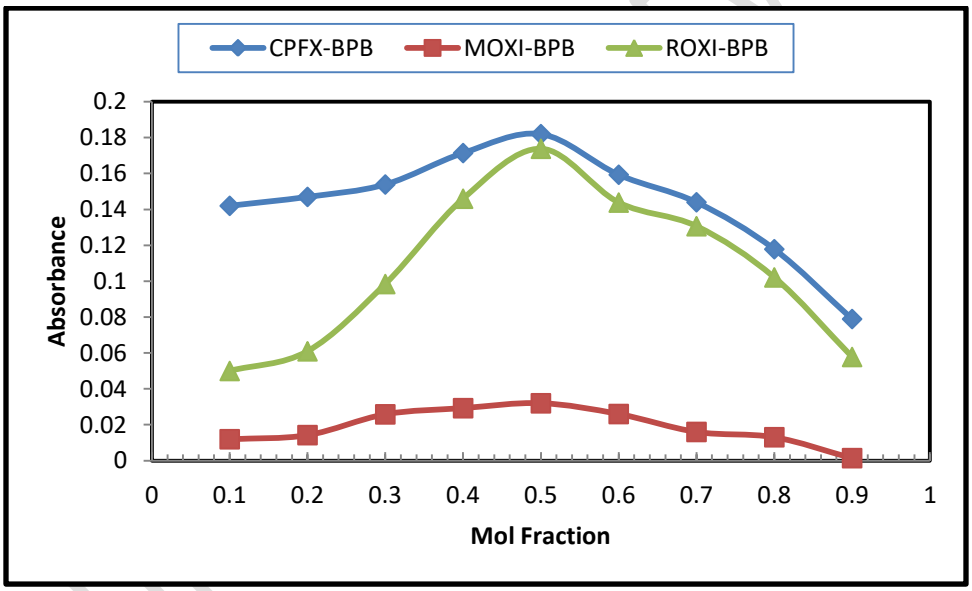

Fig. 5: Continuous variation plots for the ion pair complexes of CPFX, MOXI and ROXI (100 ppm) with BPB (2×10^{-3} M).

Analytical parameters

Calibration curves for ciprofloxacin hydrochloride, moxifloxacin hydrochloride and roxithromycin were plotted between absorbance and concentration. A linear absorbance-concentration correlation was found to be 2– 8 µg ml^{-1}, 1– 4 µg ml^{-}

[1], and 1– 6 µg $ml^{-1}$with correlation coefficients 0.9955, 0.9949 and 0.9911 respectively for CPFX, MOXI and ROXI with BPB, respectively. The limit of detection and limit of quantitation were calculated in accordance with equations,

$$LOD = 3\sigma/S$$

$$LOQ = 10\sigma/S$$

The σ is the standard deviation of the response and S is the slope of calibration graph. The detection limits were found to be 1.65, 0.83 and 0.79 µg mL^{-1}, respectively for CPFX, MOXI and ROXI with BPB. The value of correlation coefficient indicates good linearity for all the three systems.

What is the final conclusion??

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