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Spectrophotometric determination of Ciprofloxacin Hydrochloride, Moxifloxacin Hydrochloride and Roxithromycin Hydrochloride in Pure Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A straightforward, sensitive, and quick spectrophotometric approach was created and validated for the detection of Ciprofloxacin hydrochloride, Moxifloxacin hydrochloride, and Roxithromycin hydrochloride in pure form. These techniques were based upon the fact that these antibiotics produced dark yellow ion pairs when combined with bromophenol blue. 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. 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. The detection limits were found to be 1.65, 0.83 and 0.79 µg mL^{-1}, respectively, for Ciprofloxacin hydrochloride, Moxifloxacin hydrochloride, and Roxithromycin hydrochloride with bromophenol blue. The correlation coefficient value suggests that all three systems are linearly consistent.

Keywords: Ciprofloxacin hydrochloride; moxifloxacin hydrochloride and roxithromycin hydrochloride; bromophenol blue; Ion pair complex.

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1. INTRODUCTION

Ciprofloxacin hydrochloride (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. CPFX is an ideal candidate for Gastroretentive drug delivery technology. It is a broad-spectrum fluoroquinolone antibacterial agent that is predominantly absorbed from the stomach and the proximal part of the small intestine. Oral bioavailability is about 70% and reaches the peak plasma concentration 2.5 µg/ml in 1 to 2 hr after administration of 500 mg. Plasma half-life is 3-5 hours which favors the development of muccoadhesive tablets. Ciprofloxacin is an antibiotic that is useful against a wide variety of infections in human [1–5]. A fourth-generation synthetic 8-methoxyquinolone derivative of fluoroquinolone antibacterial drugs is Moxifloxacin hydrochloride (MOXI). 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 hydrochloride (ROXI) is produced from erythromycin, which has long been used to treat infections in both humans and animals In healthy volunteers, single oral dose administration of Roxithromycin 150 to 300 mg results in high, dose-related plasma concentrations of the drug; peak concentrations of 6.6 to 10.8 mg/L were recorded over this dose range within 1.5 to 1.9 hours of drug administration . 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. Herein, our work aims to determine different drugs in their pure forms such as Ciprofloxacin hydrochloride, Moxifloxacin hydrochloride, and Roxithromycin drugs, successfully using bromophenolblue as a reagent. The schematic structures for the three drugs are presented in Scheme 1.

2. EXPERIMENTAL

2.1 Apparatus

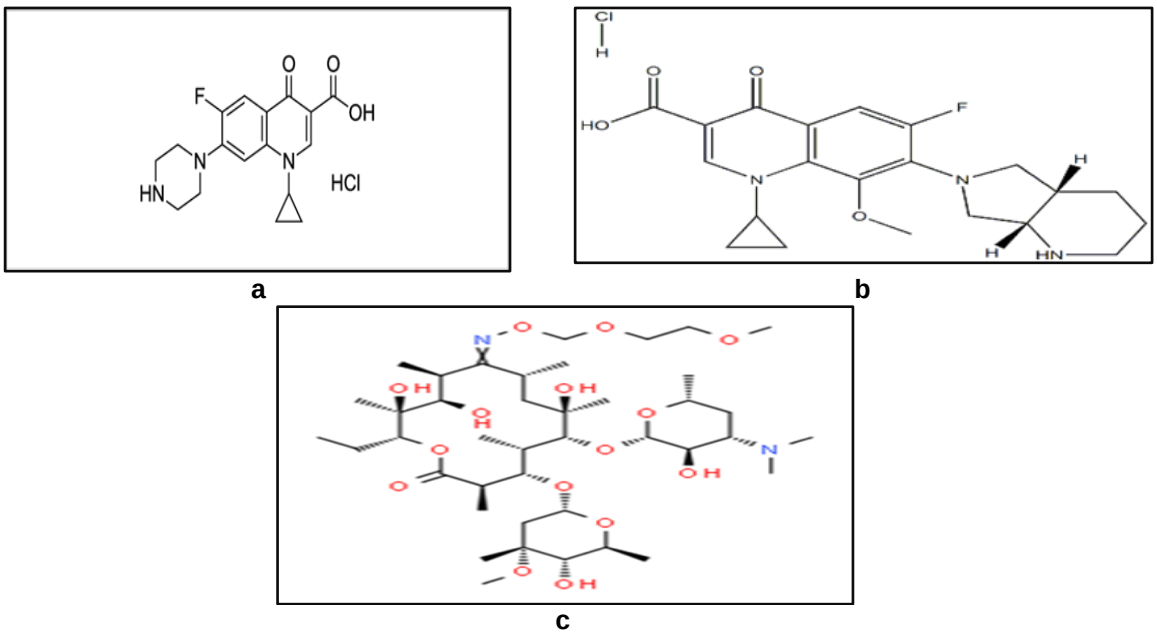
All the absorption measurements are carried out using a Jasco UV/Vis spectrophotometer (model V-670, Jasco, Tokio, Japan) with scanning speed 400 nm/min, band width 2.0 nm, and equipped with 1.0 cm pair-matched quartz cells. The pH of all solutions was adjusted using a pH-meter (HI 8014, HANNA Instruments, 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.

2.2 Materials and Reagents

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

2.3 Preparation of Solutions

Stock solutions of Ciprofloxacin hydrochloride, Roxithromycin and bromophenol were prepared



Scheme 1. Chemical structures of Ciprofloxacin hydrochloride (a), Moxifloxacin hydrochloride (b) and Roxithromycin (c)

in methanol while, Moxifloxacin hydrochloride solution was prepared in distilled water (dist. Water). The solutions were further diluted as per requirement.

2.4 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 for ciprofloxacin hydrochloride or roxithromycin solutions, and 632 nm for moxifloxacin hydrochloride against the appropriate bromophenol blue. As a reagent blank.

2.5 Procedure for Dosage Form

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 double distilled water and the same procedure were applied as described under the procedure for bulk samples.

3. RESULTS AND DISCUSSION

3.1 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 distilled water for moxifloxacin hydrochloride and methyl orange were found to be most suitable solvents.

3.2 Absorption Spectra

Solutions of ciprofloxacin hydrochloride or roxithromycin and bromophenol blue in methanol and moxifloxacin hydrochloride in distilled water 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. In experimental settings, the medication and the reagent both had little absorbance, whereas the complexes displayed maximal absorbance at these wavelengths. Hence, it was established that the investigations for quantitative analysis could be carried out at these wavelengths.

3.3 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 (Fig. 1). The optimum pH values were 5.12, 8.26 and 6.56 for CPFX, MOXI and ROXI (100 ppm) with BPB, respectively.

3.4 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 Fig. 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 and the ROXI and CPFX have stable absorbance for 30 min while MOXI lower abosorbance to reach 0 after 10 min.

3.5 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 by using 1 ml of 2mM BPB. Consequently, the calibration curve was prepared using these concentrations (Fig. 3). The means of the three values were presented in each graph after each observation was made in triplicate.

3.6 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 (Fig. 4).

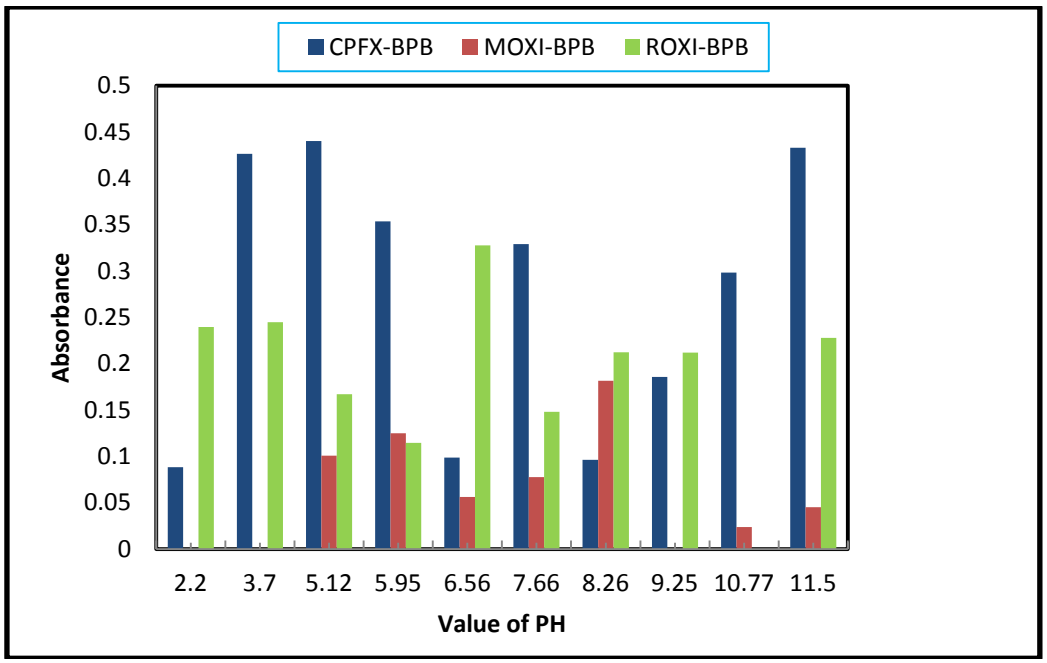


Fig. 1. Effect of pH on ion pair complexes of Ciprofloxacin hydrochloride (CPFX), Moxifloxacin hydrochloride (MOXI) and Roxithromycin (ROXI) (100 ppm) with Bromophenol blue (BPB)

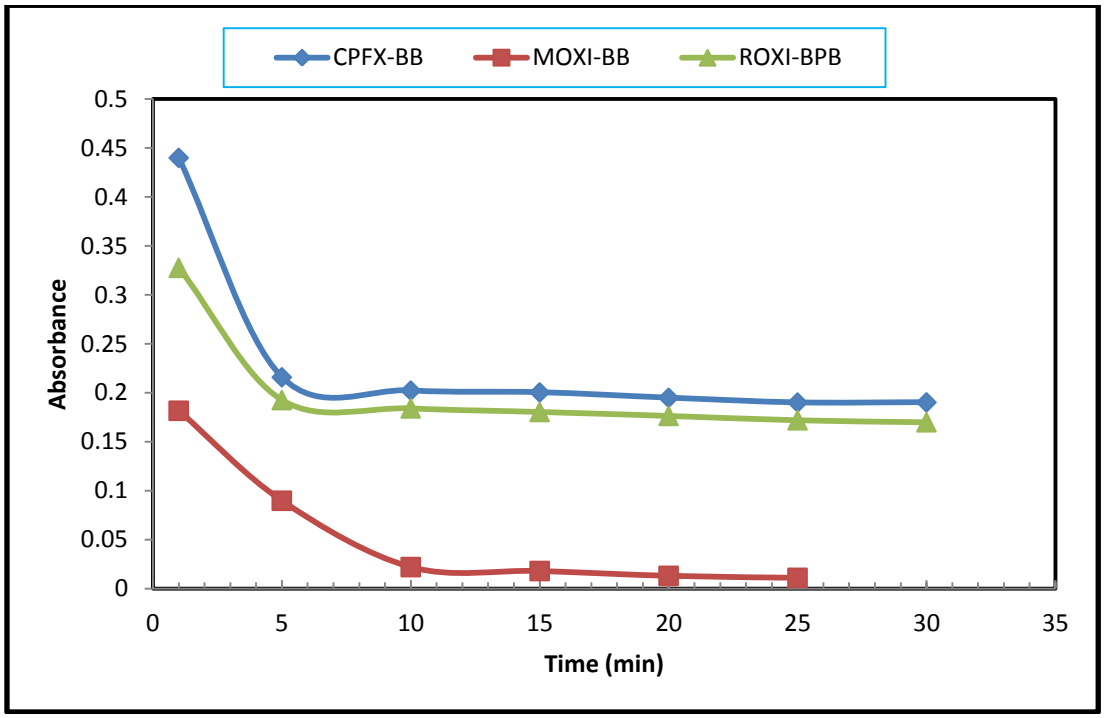


Fig. 2. Effect of time on the absorbance of ion pair complexes ciprofloxacin hydrochloride (CPFX), Moxifloxacin hydrochloride (MOXI) and Roxithromycin (ROXI) with Bromophenol Blue (BPB)

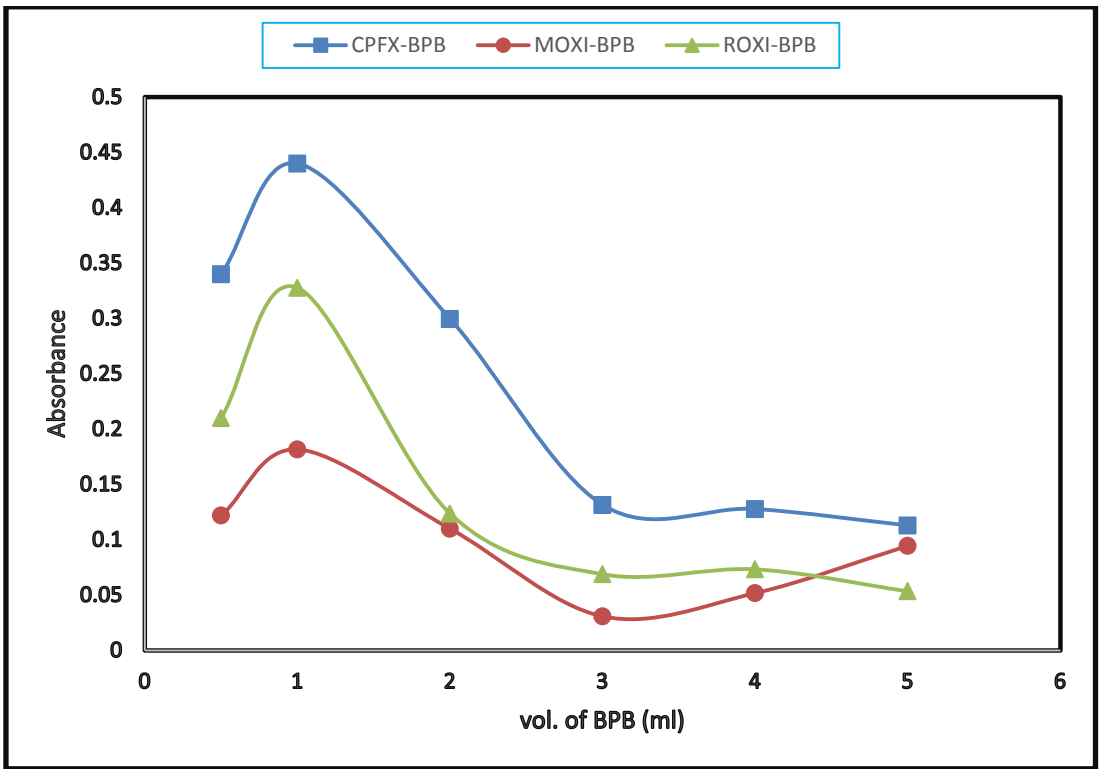


Fig. 3. Effect of Bromophenol blue (BPB) concentrations on ion pair complexes of Ciprofloxacin hydrochloride (CPFX), Moxifloxacin hydrochloride (MOXI) and Roxithromycin (ROXI) (100 ppm) with Bromophenol blue (BPB)

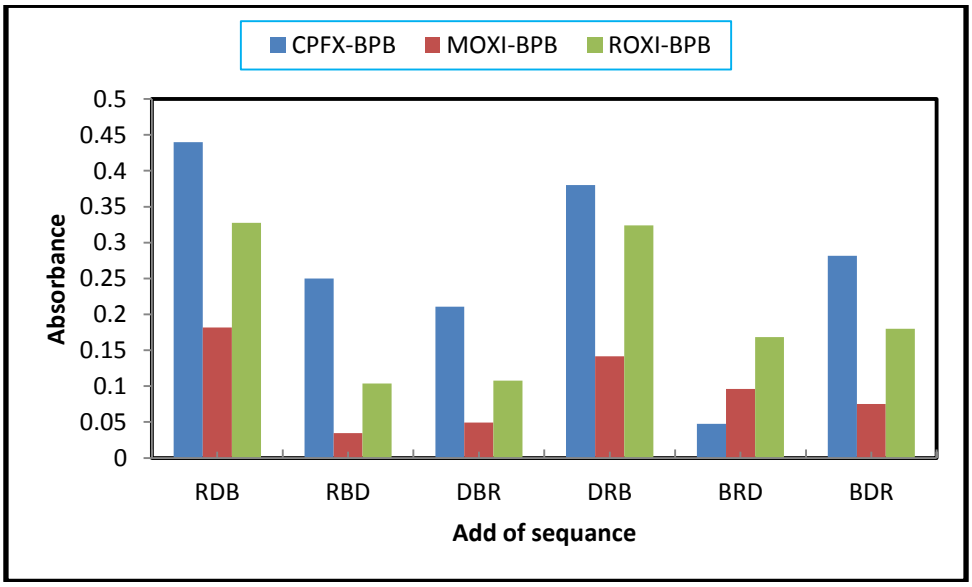


Fig. 4. Effect of sequence of addition on ion pair complexes of Ciprofloxacin hydrochloride (CPFX), Moxifloxacin hydrochloride (MOXI) and Roxithromycin (ROXI) (100 ppm) with Bromophenol blue (BPB)

3.7 Stoichiometric Relationship and Stability Studies

By using Job's continuous variation method, these complexes' composition and stability constants were determined. Different ratios of equimolar solutions of the medication and the reagent were combined, and the absorbance of each mixture was measured under ideal circumstances. The findings showed that the complexes are formed in the ratio of 1:1 (D:R) for BPB (Fig. 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.

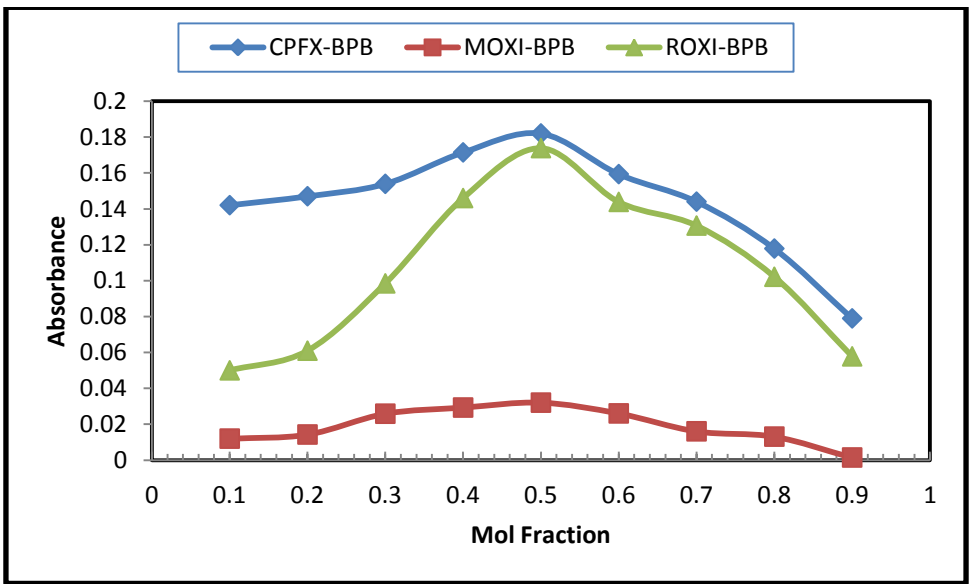


Fig. 5. Continuous variation plots for the ion pair complexes of CPFX, MOXI and ROXI (100 ppm) with BPB (2×10^{-3} M)

Table 1. The analytical parameters obtained for the formed complexes between CPFX, MOXI and ROXI with BPB

Reagent / Drug	Bromophenol blue (5×10^{-3} M) $C_{19}H_{10}Br_4O_5S$ 670 g/mol
Ciprofloxacin hydrochloride (100 ppm) $C_{17}H_{19}FN_3O_3CL$ 367.802g/mol	pH (5.12) Volume of buffer (4ml) Volume of reagent (1ml) λ_{max} (633 nm) Color of complex (dark yellow) Sequence (Reagent-Drug-Buffer) Range of Beer's law= 2– 8 µg ml^{-1} Limit of detection= 1.65 Correlation coefficient= 0.9955
Moxifloxacin hydrochloride (100 ppm) $C_{21}H_{25}CLFN_3O_4$ 437.892 g/mol	PH (8.26) Volume of buffer (4ml) Volume of reagent (1ml) λ_{max} (632 nm) Color of complex (dark yellow) Sequence (Reagent-Drug-Buffer) Range of Beer's law= 1– 4 µg ml^{-1} Limit of detection= 0.83 Correlation coefficient= 0.9949
Roxithromycin (100 ppm) $C_{41}H_{76}N_2O_{15}$ 837.058 g/mol	PH(6.56) Volume of buffer (4ml) Volume of reagent (1ml) λ_{max} (633 nm) Color of complex (dark yellow) Sequence (Reagent-Drug-Buffer) Range of Beer's law= 1– 6 µg ml^{-1} Limit of detection= 0.79 Correlation coefficient= 0.9911

3.8 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 (Table 1). The value of correlation coefficient indicates good linearity for all the three systems.

4. CONCLUSION

The spectrophotometer is easy to use and inexpensive, in contrast to gas chromatography and HPLC methods. The significance is not in the instrument's complexity, but rather in the chemical processes that underlie the methods. The ability to evaluate a specific component in complicated dose makes this element of spectrophotometric analysis of great interest to analytical pharmacists. The procedures don't require any difficult sample preparation or crucial reaction conditions, and the reagents used in the proposed methods are less expensive and more easily accessible. Small changes in experimental parameters like pH and reagent concentration have no impact on the procedure. Additionally, the procedures are devoid of influence from typical excipients and additives, accurate,

reproducible, and sensitive enough. The assay of CPFX, MOXI, and ROXI in pure form has successfully demonstrated the broad applicability of the novel methodologies for routine quality control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. El-Hawwary A, Sarhan N. Does Olive Oil Attenuate Ciprofloxacin-Induced Renal Cortical Toxicity? Light and Electron Microscopic Study. Egyptian Journal of Histology. 2017;40(2):253-64.
2. Vanithakumari G, Priyadharshin KM. Ciprofloxacin induced hormonal changes in the thyroid gland of rats and Anti-oxidant vitamin A, C and E as rescue agents. J P B S. 2013;5(3):12-18.
3. Zobeiri F, Sadrkhanlou RA, Salami S, Mardani K, Ahmadi A. The effect of ciprofloxacin on sperm DNA damage, fertility potential and early embryonic development in NMRI mice. Vet Res Forum. 2012;3(2):131–135.
4. Koziel R, Zablocki K. Calcium signals are affected by ciprofloxacin as a consequence of reduction of mitochondrial DNA content in Jurkat cells. Antimicrob Agents Chemother. 2006;50:1664-1671.
5. Sen S, Jaiswal AK, Yanpallewar S, Acharya SB. Anxiogenic potential of ciprofloxacin and norfloxacin in rats. Singapore Med J. 2007;48(11):1028-1032.
6. Balboul BA, El-Roudia AM, Mohameda ES, Derayeab SM, Abdelmageedb OH. Synthesis, Characterization, Spectrofluorometric and Antibacterial Activity Studies of Moxifloxacin-Zirconium Complex. Egyptian Journal of Chemistry. 2012;55(1):15-31.
7. Sullivan JT, Woodruff M, Lettieri J, Agarwal V, Krol GJ, Leese PT, Watson S, Heller AH. Pharmacokinetics of a once-daily oral dose of moxifloxacin (Bay 12-8039), a new enantiomerically pure 8-methoxy quinolone. Antimicrobial agents and Chemotherapy. 1999;43(11):2793-7.
8. Mitscher LA, Devasthale P, Zavod R, Relationships SA. Quinolone Antimicrobial Agents. American Society for Microbiology, 2nd Edi., Washington DC. 1993;3-53.
9. Andriole VT. Overview of the fluoroquinolones: focus on moxifloxacin. Formulary. 2002;37:13.
10. Ni BJ, Zeng S, Wei W, Dai X, Sun J. Impact of roxithromycin on waste activated sludge anaerobic digestion: methane production, carbon transformation and antibiotic resistance genes. Science of the Total Environment. 2020;703:134899.
11. Davis R, Brogden RN, Markham A, Faulds D. Erratum to: Roxithromycin: an update of its antimicrobial activity, pharmacokinetic properties and therapeutic use. Drugs. 1994;48(5):793.
12. Young RA, Gonzalez JP, Sorkin EM. Roxithromycin. Drugs. 1989;37(1):8-41.
13. Puri SK, Lassman HB. Roxithromycin: a pharmacokinetic review of a macrolide. Journal of Antimicrobial Chemotherapy. 1987;20(suppl_B):89-100.
14. Kümmerer K. Antibiotics in the aquatic environment–a review–part I. Chemosphere. 2009;75(4):417-34.
15. Dangui AZ, Santos VM, Gomes BS, de Castilho TS, Nicolini KP, Nicolini J. Preferential solvation bromophenol blue in water-alcohol binary mixture. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2018;203:333-41.
16. Shokrollahi A, Zare E. Determination of acidity constants of bromophenol blue and phenol red indicators by solution scanometric method and comparison with spectrophotometric results. Journal of Molecular Liquids. 2016;219:1165-71.
17. Janzen MC, Ponder JB, Bailey DP, Ingison CK, Suslick KS. Colorimetric sensor arrays for volatile organic compounds. Analytical chemistry. 2006;78(11):3591-600.
18. Feng L, Musto CJ, Kemling JW, Lim SH, Zhong W, Suslick KS. Colorimetric sensor array for determination and identification of toxic industrial chemicals. Analytical Chemistry. 2010;82(22):9433-40.
19. Morita T, Assumpção R. Manual de soluções, reagentes e solventes: padronização, preparação, purificação, indicadores de segurança e descarte de produtos químicos. Editora Blucher; 2007.
20. Rodríguez AJ, Zamarreño CR, Matías IR, Arregui FJ, Domínguez Cruz RF, May-Arrioja DA. A fiber optic ammonia sensor using a universal pH indicator. Sensors. 2014;14(3):4060-73.

21. Gouda AA, Amin AS, El-Sheikh R, Yousef AG. Spectrophotometric determination of gemifloxacin mesylate, moxifloxacin hydrochloride, and enrofloxacin in pharmaceutical formulations using acid dyes. Journal of Analytical Methods in Chemistry. 2014;2014.
22. Ray D, Ha KC, Nie K, Zheng H, Hughes TR, Morris QD. RNAcompete methodology and application to determine sequence preferences of unconventional RNA-binding proteins. Methods. 2017;118:3-15.
23. Dole T, Koltun S, Baker SM, Goodrich-Schneider RM, Marshall MR, Sarnoski PJ. Colorimetric evaluation of mahi-mahi and tuna for biogenic amines. Journal of Aquatic Food Product Technology. 2017;26(7):781-9.
24. Silveira TR, Ebling CD, Dal Magro L, Rodrigues RC, Schneider WD, Camassola M, de Menezes EW, Meneguzzi Á, Klein MP. An efficient decolorization of methyl orange dye by laccase from Marasmiellus palmivorus immobilized on chitosan-coated magnetic particles. Biocatalysis and Agricultural Biotechnology. 2020;30: 101859.
25. Sen SK, Raut S, Bandyopadhyay P, Raut S. Fungal decolouration and degradation of azo dyes: a review. Fungal Biology Reviews. 2016;30(3):112-33. Available:https://doi.org/ 10.1016/j.fbr.2016.06.003.
26. Zofair SF, Arsalan A, Khan MA, Alhumaydhi FA, Younus H. Immobilization of laccase on Sepharose-linked antibody support for decolourization of phenol red. International Journal of Biological Macromolecules. 2020;161:78-87. Available:https://doi.org/10.1016/j. ijbiomac.2020.06.009.
27. Zhang B, Wang Z, Zhou X, Shi C, Guo H, Feng C. Electrochemical decolorization of methyl orange powered by bioelectricity from single-chamber microbial fuel cells. Bioresource Technology. 2015;181:360-2. Available:https://doi.org/10.1016/j. biortech.2015.01.076.
28. Gainza AH, Konyeaso RI. Substitution reactions of benzethonium chloride with ion associates of bromocresol green–quinine and bromophenol blue–quinine in dichloromethane. Canadian Journal of Chemistry. 1991;69(6):937-44.

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