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Spectrophotometric Determination of Moxifloxacin HCl in Pure and Blood Sample

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ABSTRACT

A reaction between Moxifloxacin HCl and $FeCl_3$ solution in slightly acidic medium was studied. It gave brown colour complex having maximum absorbance at 407.0nm. The reaction is selective for Moxifloxacin HCl with 0.00024µg/uL and different concentration of FeCl3 was applied. The color reaction obeyed Beer's law from 0.01 µg /µL to 0.00018 µg/µL of $FeCl_3$ solution having concentration 0.1ug/uL and relative standard deviation 0.026**%.**The quantitative estimation of moxifloxacin HCl in blood sample is also studied.

Key Words: Moxifloxacin, UV-Visible Spectrophotometer, Ferric Chloride, Beer's law, Color reaction, Analytical method

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INTRODUCTION

Hydrochloride ($C_{21}H_{24}FN_3O_4$.HCl) belongs to the fluoroquinlones. Fluoroquinolones (Figure 1) are totally synthetic and closely related structurally to an earlier oldest quinolones i.e; Nalidixic Acid. Moxifloxacin is a broad spectrum antibiotic and has vitro activity against a wide range of gram positive, gram negative and also against β-lactum bacteria[1]. It was found that *Staphylococcus epidermidis* strain has endophthalmitis and corneal ulcers resistant against the Moxifloxacin[2]. (Systematic (IUPAC) Name: 1-cyclopropyl-7-[(1*S*,6*S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo- quinoline-3-carboxylic acid)

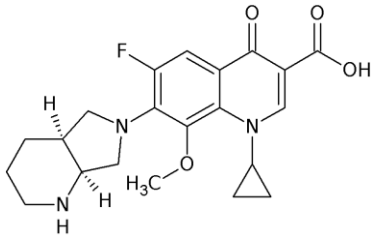

Figure I Structure of Moxifloxacin Hydrochloride

There are various analytical techniques used for the determination of drug in the blood and pharmaceutical samples. Analysis may be carried out by using color developing assays. Color developing assay may utilize the organic or inorganic regent[3]. A voltammetric method is reported for the determination of moxifloxacin in tablets by the formaton of moxi-copper complex[4]. Spectrophotometric method includes the reaction of fluoroquinolones with Brilliant Blue G (BBG) in NaOAc-AcOH buffer of pH 4.0 for CPF and NRF or in neutral medium for MDH and IPH to give chloroform soluble ion-association complexes[5]. Another method reported with Supracene Violet 3B (SV 3B, method A) and tropaeolin 000 (TP 000, method B) are described[6]. Spectrophotometric, atomic absorption and spectrofluorimetric method also used by using the cobalt sulphate as ion pair complex[7]. Similar method is applied by forming the blue color complex with cobalt tetrathiocyanate[8]. Xi Gen Huang reported the simultaneous determination of nofloxain, ofloxacin and lmoefloxacin with the aid of chemometric method[9].

Spectrofluorimetric method allows the determination of 30-300 ng/ml Moxifloxacin in an aqueous solution containing phosphoric acid-phosphate buffer (pH 8.3) with with λexc = 287nm and λem=465nm. Detection and quantification limit were 10 and 30 ng/ml respectively with a relative standard deviation (n=10) of 2%.This method was applied to determination of moxifloxacin in three spanish commercial pharmaceutical formulations[10]. Present study focus on the development of cheaper and accurate method for the determination of Moxifloxacin HCl in pharmaceutical and blood samples.

MATERIALS AND METHODS

Apparatus

A UV-VIS spectrophotometer (Techcomp-UV2300) was used to measure the absorbance. A pH meter (Cyber Scan) and graduated pipettes were employed.

Reagent:

Analytical grade reagents are used for whole study. Moxifloxacin and $FeCl_3$ obtained from E. Merck. Standard solution of Moxifloxacin HCl (w/v) 0.00024µg /uL was prepared in doubly distilled water by dissolving 0.096g of Moxifloxacin HCl in 10.0ml distilled water.

Then taking 1.0mL of prepared solution and diluted it up to 400.0mL 1 % (w/v) solution of $FeCl_3$ prepared by dissolving 1.0 g of it in 20 mL of doubly distilled water and makes the volume up to 100mL using distilled water. Final concentration of $FeCl_3$ is 1.0 µg/µL. Ferric ion is extremely prone to hydrolysis and few drops of HCl must be added in stock solution to prevent hydrolysis.

General Procedure

To an aliquot of Moxifloxacin HCl, added 10 µml of $FeCl_3$, brown color complex was formed. Scanning of the complex was taken from the 250 to 800 nm. The absorbance of the resulting brown complex color was at 407.0 nm. Subsequently performed different effects including concentration, pH, time, temperature and blood sample. A calibration curve was drawn for each effect taking 5.0mL Moxifloxacin HCl each time with different concentration of $FeCl_3$.

Validity on Blood Sample

Took blood sample in anticoagulant tube and centrifuge it at 4000 rpm for 5 minutes. Serum was separated from the blood and divided in to eight portions (vials), each portion contain 0.5ml serum. Then 3 mL acetonitrile was also added in each of the tube[11]. Centrifuge them at 5000 rpm for the removal of proteins. Then added 5ml Moxifloxacin HCl solution in each tube and different concentrations of reagent ($FeCl_3$ solution i.e. 20µL, 40µL, 60µL, 80µL, 100µL, 120µL, 140µL, 160µL) in each vial with a difference of 20µl of $FeCl_3$, shake well and measure the absorbance at 407nm.

RESULTS AND DISCUSSION

Absorption Spectrum of Colored Complex

Moxifloxacin HCl reacts with $FeCl_3$ without any heating i.e, at 30°C, giving brown colour Complex, having maximum absorbance at 407.0 nm. Scientist used this ion pair complex as a quantitative tool for the analysis of drug. Moxifloxacin found to react with the $FeCl_3$ to form ion-

pair complex in acidic medium.

Effect of Color Producing Reagent

Color developing assay is based upon the formation of complex between moxifloxacin and Iron. For the development of color Ferric chloride was used. It was found that Stable complex was produced and increasing concentration of $FeCl_3$, absorbance also increases thus linear relation observed. This shows that complex formation reaction obeys Beer's law (Figure II).

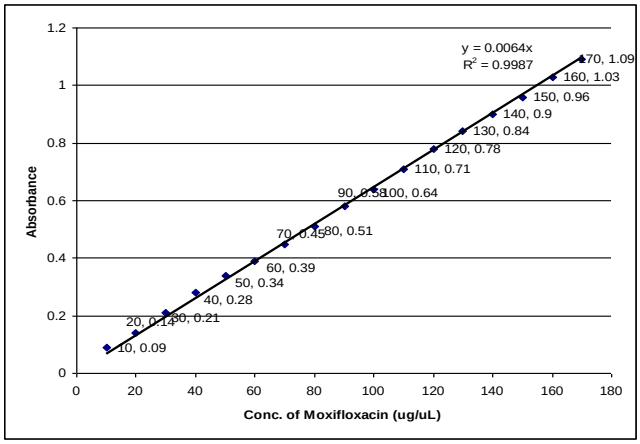

Figure II Conditions.$FeCl_3$ 0.1×10^{-3}µg/µL Moxifloxacin HCl 0.24µg /µL, reaction temp. 30°C, pH = 5.11, λ_{max} 403nm

Effect of pH

When Moxifloxacin HCl solution was mixed with $FeCl_3$ solution, 5ml and 150µl respectively, a brown complex was obtained without the addition of NaOH and the pH was 2.81. However, by the addition of NaOH the intensity of the color is enhances and absorbance should also increases. This increase in the color intensity refers towards the completion of the chemical reaction between reacting molecules. Maximum absorbance was obtained at pH 5.11 (Figure III).

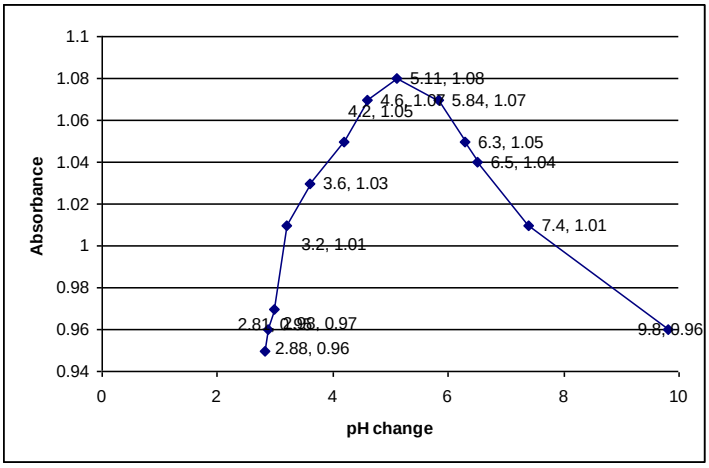

Figure. III Effect of pH .

Effect of Temperature

The effect of temperature is shown in The colour develops at room temperature. As the temperature increases up to 100°C the complex did not dissociate and solvent evaporate due to which the colour intensity increase, the complex is stable by heating up to 100°C and colour was stable (Figure IV). Conditions. $FeCl_3$ 150.0µL with 0.1×10^{-3}µg/µL, Moxifloxacin HCl 5.0 mL with 0.24µg/mL

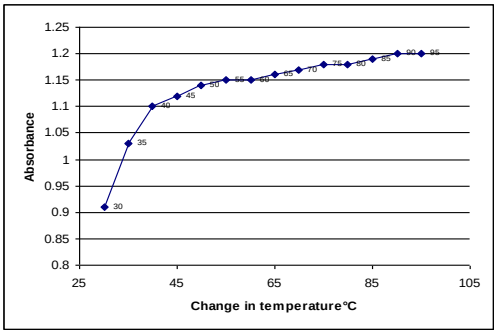

Figure. IV Effect of temperature on the stability of complex

Effect of Heating Time

The optimum temperature for this color reaction is 100°C. Color develops at room temperature but when the complex temperature is maintained at 100°C ,observe for different interval of time with a difference of 5 min. and measure the absorbance at 407nm it increase. Off course reaction takes place at 100°C so there is no effect of the heating time on the chemical reaction as temperature uniform and there was no any chemical change took place. But the color intensity increase due to evaporation of water molecules which will increase the concentration and absorption increases. (Figure V). Conditions $FeCl_3$ 150.0µL with 0.1×10^{-3}m.g/ µL , Moxifloxacin HCl 5.0 mL with 0.24µg/mL , temp. 100°C

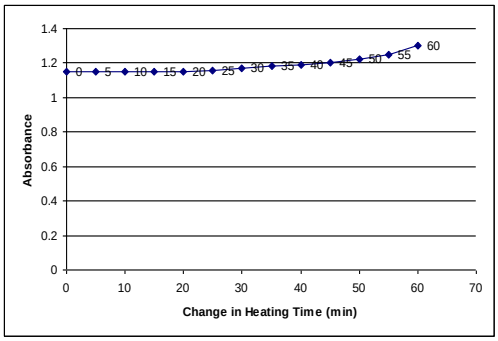

Figure V :Effect of heating time.

Validity on Blood Sample:

Acetonitrile was used for the removal of proteins to increase the visibility during the spectro-photometric analysis, which can decrease the chances of error. The results proved that the

method could be applicable on biological samples and an excellent method for determination of 0.00024 µg/µL Moxifloxacin HCl by using 140µL of $FeCl_3$. Results expressed in Figure VI and VII.

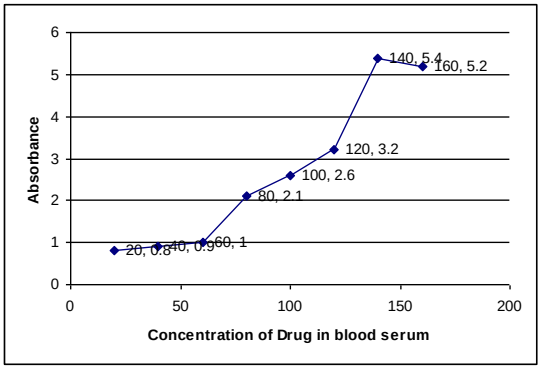

Figure. VI: Conditions.$FeCl_3$ 0.1×10^{-3}mg/µL Moxifloxacin HCl 0.24µg /mL, reaction temp.30°C

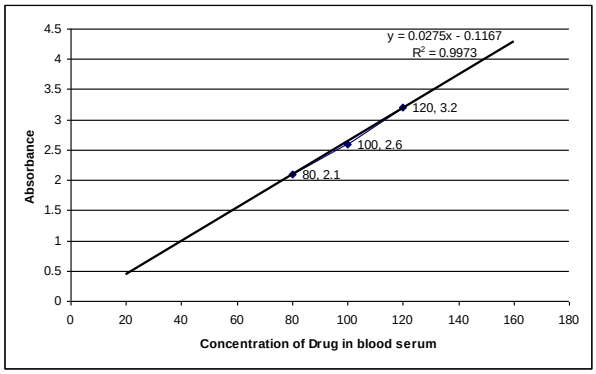

Figure. VII Concentration of Moxifloxacin HCl in blood sample

Sensitivity

The method was employed for the determination of moxifloxacin HCl in the different solution samples to check the sensitivity and reproducibility (Table-1).

Table-1:Determination of Moxifloxacin HCl from Pure Solution

Moxifloxacin HCl taken (µg/µL)	Moxifloxacin HCl found (µg/µL)	RSD (%)
0.05	0.0501	0.014
0.1	0.1001	0.012
0.15	0.1496	0.026
0.2	0.2002	0.017
0.25	0.2503	0.018
0.3	0.3002	0.01
0.35	0.3505	0.019
0.4	0.4002	0.018
0.45	0.4503	0.023
0.5	0.4996	0.022

Table-II: Determination of Moxifloxacin HCl

Parameter	Value
λmax (nm)	407.0 nm
Beer's law limit (µg /uL)	10.0-180
Limit of detection (µg /uL)	0.01
Optimum photometric range (µg /uL)	0.01-0.00018
Relative standard deviation (RSD) (%)	0.026

CONCLUSION

Pharmaceutical drug sample was prepared in the range of 0.05 to 0.5µl. Absorbance of the complex was determined at 407.0 nm. Method is precise and accurate, because the amount taken from the identical samples is known and the amount found does not exceed the relative standard deviation of 0.26 % for seven values. The optimization has been done at lower analytical concentration. All the findings are reported in Table-II.

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