SPECTROPHOTOMETRIC ESTIMATION OF MOXIFLOXACIN HYDROCHLORIDE USING OSMIUM(OS-VIII) IONS IN MICELLAR MEDIUM

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A simple ,sensitive and accurate spectrophotometric method was described for the determination of Moxifloxacin hydrochloride (MFX) a broad spectrum flouroquinolone anti bacterial either in pure form or in the tablet. The method is based on chelate formation between MFX and osmium (Os VIII) in aqueous media at P_H 8 in presence of surfactant. The complex showed an absorption maximum at 368nm for zero order, Ist derivative at 435 nm and Second derivative at 454 nm respectively with apparent molar absorpitivity of 2.1867×10^4 L-M^{-1} cm^{-1}, and sandell's sensitivity of 0.061 µg/cm^2 respectively. The solution of the complex obeyed beer's law in the concentration range of 0.5-5 µg/ml for zero order, 1 to10 µg/ml for Ist order and 1to 15 µg/ml for 2nd order respectively. The limit of detection and Limit of quantification were calculated and RSD were calculated. The chelate composition between MFX and Os (VIII) ion was found to be 1 : 1 ratio determined by Job's continuous method and by Molar ratio method. The proposed method was applied for the determination of MFX in tablets without interference from common excipients .The results obtained by the application of this procedure showed percentage recoveries were 100.01 ± 0.0192 for zero order, 100.2 ± 0.1463 for Ist order and 100.3 ± 0.1065 for 2nd order respectively.

Flouroquinolone / Moxifloxacin / Hydrochloride / Chelate / Aqueous media / Spectrophotometric / Pharmaceutical formulation

INTRODUCTION

Moxifloxacin (MFX) chemically 1-cyclopropyl-7-((s, s)-2, 8-diazabicyclo (4.3.0) non-8-yl)-6-Fluoro-8-methoxy-1, 4-dihydro-4-oxo-3quinoline carboxylic acid, A new flouroquinolone antibacterial compound Moxifloxacin can be used to treat respiratory infections, including acute sinusitis, acute exacerbations of chronic bronchitis, and community- acquired pneumonia, as well as skin and skin structure infections. Moxifloxacin is used as a second-line agent in tuberculosis (TB) and may potentially have benefits in reducing treatment duration from its current six month to four months. In ophthalmology, Moxifloxacin is available in the form of eye drops, to treat conjunctival infections caused by susceptible bacteria and to prevent infection following eye surgeries [1, 2]. Only few methods for the analysis of MFX are available which include derivative spectroscopy in micellar medium [3], simple UV spectrophotometric methods [4, 5], HPLC methods [6-11], HPTLC [12-13], LC/MS/MS [14, 15], Differential pulse polarography [16], capillary electrophoresis [17]. Complexes of drugs with Ruthenium (III) have also been studied such as with antitumour drugs. The purpose of this present study was to develop direct and derivative spectrophotometric, stability indicating procedure for the selective determination of MFX by chelation with Os(VIII) ions, to develop procedure capable of quantitation, describe and validate the structural ability of MFX to chelate with Os(VIII). The methods based on chelation of drug with osmium have not been studied and prospective work will be the study using proposed chelation procedures by direct and derivative spectroscopy, is a useful technique that resolves two overlapping spectra and eliminating matrix interference in the mixture of components [18-20].

EXPERIMENTAL

Apparatus. All absorption Spectra were made using SCHIMADZU-160 A U.V-VIS Spectrophotometer equipped with 10mm matched Quartz cells.

Materials and reagents. Osmium (VIII) solution: The standard solution of osmium(VIII) was prepared by dissolving 1.0 gm of osmium tetra oxide in 100 ml of 5N H_2SO_4 and diluted to 50 ml with distill water. The stock solution was standardized spectrophotometrically [21].

MFX 1×10^{-3} M solution was prepared by dissolving 43.8 mg of MFX into a 100ml volumetric flask. It was dissolved and diluted upto the mark using double distilled water and sodium dodecylsulphate (1.2 ml of 0.1 M) to get 1×10^{-3} M concentration of MFX solution.

Chelation of Mfx with Os(VIII):s

The following procedure was adopted for measuring the absorption spectra of complex (metal + drug) in aqueous medium. In a 10-ml standard flask, 1ml of 1×10^{-3} M MFX stock solution the metal complex was prepared by taking 3 ml of buffer, suitable volume of surfactant, suitably concentration of Osmium ion metal solution (usually 10-15 fold molar excess to drug) solutions. The contents were diluted up to the mark with distilled water and the absorbance of the complex was measured against the reagent blank prepared identically. A plot between absorbance and the wavelength was plotted from which the analytical wavelength was selected. The ƛ-max for the Chelate at Zero order is 368 nm, first order derivative is obtained at 435 nm and second order derivative at 454 nm. Figs 1-3.

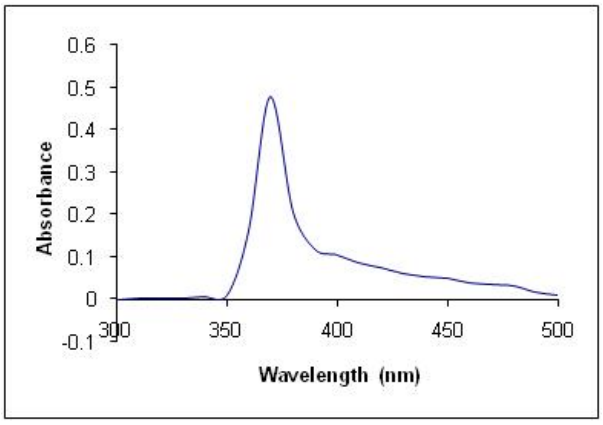

Fig. 1. Absorption spectra of 43.8µg/ml MFX complex with 1×10^{-2} M Os (VsIII).

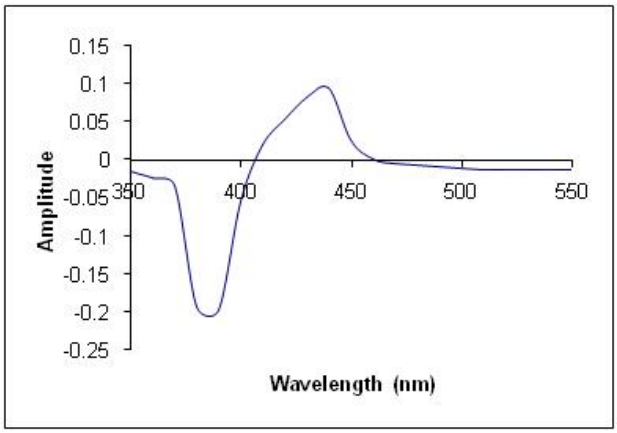

Fig. 2 First order derivative spectra of 43.8 µg/ml MFX complex with 1×10^{-2} M Os(VIII).

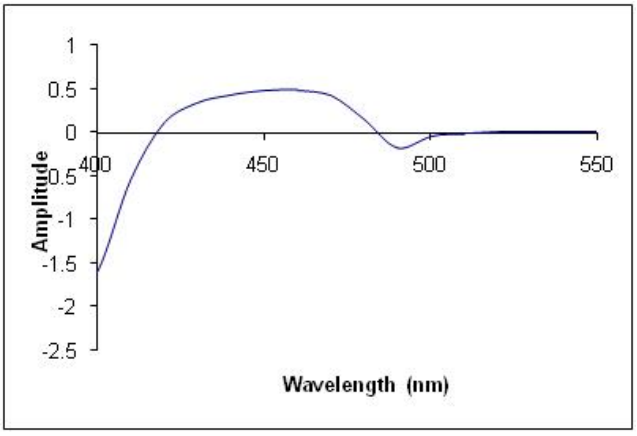

Fig. 3. Second order derivative spectra of of43.8 µg/ml MFX complex with 1×10^{-2} M Os(VIII).

Procedure for dosage form

An accurately weighed amount of finely powdered tablet equivalent to 100 mg of drug was dissolved in about 10ml of distilled water and transferred in to 100ml of calibrated

volumetric flask and 1.2 ml of 0.1 M sodium dodecyl sulphate was added, after 15 minutes mechanical shaking was filtered into a 100 ml of calibrated volumetric flask through Whatmann no : 41 filter paper, diluted to 100ml with distilled water and the same procedure was followed as described above.

Optimum conditions

Effect of pH. To arrive the optimum pH required for achieving the maximum and constant absorbance, the effect of pH on the absorbance of the Osmium (VIII)–MFX complex was studied by employing in a set of 10-ml standard flasks, 3 ml of buffer (different pH values 1.0 to 11.0) solution, constant amount of drug and metal ion (usually 10-15 fold molar excess to drug) solution were taken, made up to the mark with distilled water. The absorbance of each solution (metal complex) was measured at a selected wavelength (λ_{max}) against corresponding reagent blank prepared accordingly. A plot was made between absorbance and pH from which the working pH was selected.

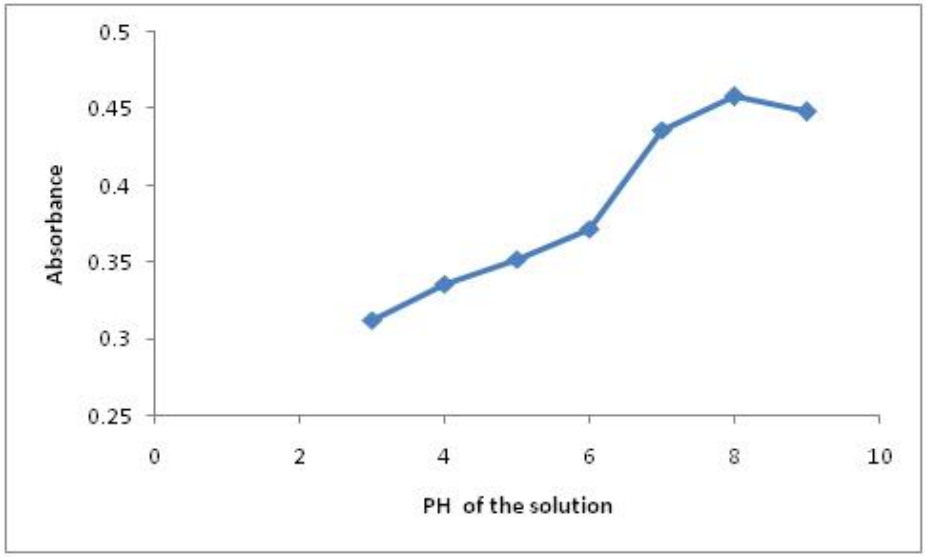

Fig. 4. Effect of P_H on the formation of MFX complex with Os (VIII) ion.

The complex shows maximum and constant absorbance in the pH range 8.0. Therefore, buffer solution having pH 8.0 was chosen for further studies Fig 4.

Effect of reagent concentration. To 1ml of 1×10^{-3} M MFX stock solution, aliquots of 0.5 to 3 ml of 1×10^{-2} M reagent solution (Os VIII) was added into 10 ml Volumetric flask and make upto the volume to 10ml with distilled water and the absorbance values at 368 nm. Investigation of metal ion concentration revealed that only ten-fold molar excess of reagent was sufficient for optimum and maximum colour intensity of the chelate of MFX using 43.8 µg/ml concentration Fig 5.

Effect of time. The absorbance of MFX-Os(VIII) complex was measured at different time intervals to ascertain the time stability of the complex. The full colour development of the complex remains constant for twenty four hours. Then the absorbance of MFX-Os (VIII) complex was measured at 368 nm.

Determination of chelate stability and composition. The composition of the chelate [21-26] of MFX with Os(VIII) ion used was studied by Job's continuous method and Molar

ratio method. The chelate of 1 : 1 ratio was obtained between MFX and Os(VIII) Fig. 6 and Fig. 7.

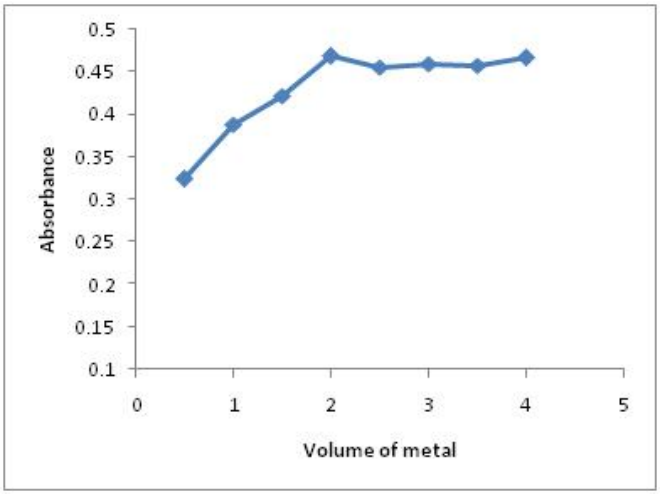

Fig. 5. Effect of reagent concentration on the formation of MFX complex with Os (VIII) ion.

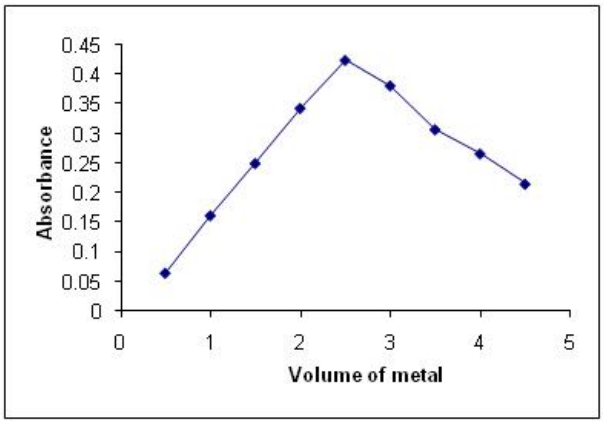

Fig. 6. Job's method for MFX complex with Os (VIII) ion at 368 nm.

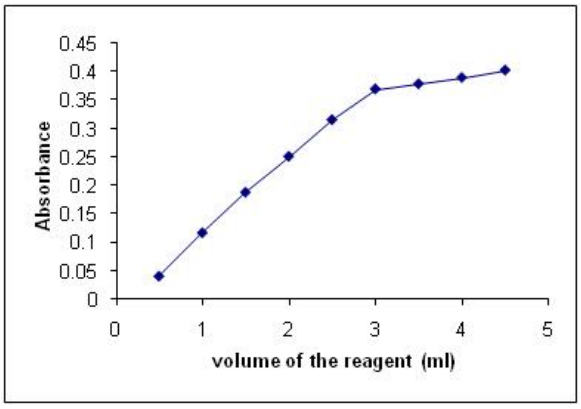

Fig. 7. Mole ratio method for MFX complex with Os (VIII) ion at 368 nm.

The stability constants of formed chelate were calculated and the values of Log β was 6.55.The results were tabulated in Table 1.

Table 1. Stability constants of Moxifloxacin hydrochloride chelate with Osmium ion by Job's method.

Parameters	MFX-Os(VIII) λmax at 368nm
Total molar conc	1×10^{-5}M
N	2.303
β*	3.61×10^{6}
Log β	6.55

Linearity range and quantification procedure. Beer's law was found to be obeyed in the concentration range of 0.5to 5 µg/ml for Zero order, 1 to 10 µg/ml for Ist order derivative and 1to 15 µg/ml for 2nd order derivative. A (1%, 1 Cm) was calculated. The results were tabulated in Table 2.

Table 2. Results of validation.

Parameter	MFX-Os(VIII) 368 nm	MFX-Os(VIII) Ist derivative	MFX-Os(VIII) 2nd derivative
Linearity range(µg/ml	0.5-5	1-10	1-15
LOD(mcg/ml)	1.34	0.27	0.12
LOQ(mcg/ml)	4.068	0.81	0.384
Slope	0.005	0.023	0.038
Intercept	0.104	0.009	0.026
Correlation coefficient	0.999	0.999	0.997
Accuracy	100.01	100.2	100.3
Repeatability(n = 6)	0.0192	0.1065	0.1581

Assay of dosage form [27-36]. An accurately weighed amount of finely powdered tablet equivalent to 100mg of drug was dissolved in about 10ml of distilled water and transferred in to 100ml of calibrated volumetric flask, 1.2 ml of 0.1 M sodium dodecyl sulfate was added and after 15 minutes of mechanical shaking was filtered into a 100ml of calibrated volumetric flask through Whatmann no : 41 filter paper and was diluted to 100ml with distilled water and the same procedure was followed as described above. The results were tabulated in Table 3.

Table 3. Results of the determination of MFX by the proposed method in their dosage form.

Dosage Form	MFX-Os(VIII) At 368nm	MFX-Os(VIII) At 435	MFX-Os(VIII) At 459	LIMIT
Tablet 1	100.03 ± 0.1105 N = 6	100.11 ± 0.1342 N = 6	100.20 ± 0.1064 N = 6	The Assay of Moxifloxacin Hydrochloride tablets should be within 98%-102%
Tablet 2	100.03 ± 0.1246 N = 6	100.20 ± 0.1066 N = 6	100.20 ± 0.1152 N = 6	

Interference study. Potential interference by the excipients in the dosage form was also studied, samples were prepared by mixing fixed amounts of common excipients such as lactose, Micro crystalline cellulose, Talc, Magnesium sterate and Starch. The good percentage recoveries were obtained indicating no interference was observed. The results were tabulated in Table 4.

Table 4. Determination of Moxifloxacin in presence of common excipients by the proposed method.

Excipient	Recovery ± RSD At 368nm	Recovery ± RSD 1st derivative At435 nm	Recovery ± RSD 2nd derivative at 454 nm
Lactose(10mg)	100.01 ± 0.1067	100.15 ± 0.1705	100.23 ± 0.1371
Talc(10mg)	100.05 ± 0.0956	100.15 ± 0.1488	100.25 ± 0.1380
Magnesium sterate(10mg)	100.03 ± 0.1105	100.20 ± 0.1152	100.30 ± 0.1628
Starch(10mg)	100.05 ± 0.1257	100.15 ± 0.1256	100.30 ± 0.1339
Microcrystalline cellulose	100.00 ± 0.1	100.20 ± 0.1341	100.20 ± 0.1150

(a) Values are mean of six determinations.

Results and discussions

The linearity range of MFX-Os(VIII) chelate covered over a range of 0.5-5 µg/ml of drug with A(1%,1cm)equals to 2.1867×10^4 L $Mole^{-1}$ cm^{-1}. The drug chelate absorbance were plotted against the corresponding concentrations. Data were fitted to the equation Y = a + bx, where Y is the absorbance at relevant maximum is the Drug concentration in mcg/ml; b is the slope and a is the intercept of the calibration curve. The correlation coefficient is 0.999 indicating exact linearities. The Accuracy of the proposed procedure were 100%. Repeatability and reproducibility were evaluated. Proposed procedure for MFX is a stability indicating one which can be used for the determination without interference with the excipients. The drug being soluble in presence of surfactant in aqueous medium and considered more selective drug to chelate with Os (VIII) ion, in addition, the derivative spectra normally contain more apparent spectral details than the normal spectra, more selective and sensitive in eliminating the background interference of complex matrix in resolving individual drug, drug additives and drug decomposition both interfered.

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