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Spectrophotometric Methods for the Determination of Ofloxacin Hydrochloride in Pure and Pharmaceutical Formulations

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Abstract: Three simple, sensitive, rapid and accurate colorimetric methods (method I, II, and III) have been developed for the estimation of ofloxacin hydrochloride (OFH) in bulk and pharmaceutical dosage forms. Method I and II are based on ion-pair complex formation between ofloxacin hydrochloride and erichrome black T and orange G in acidic buffer solution followed by their extraction in organic solvent (chloroform).The absorbance of the organic layer was measured at their respective wavelength (512 nm for method I and 484 nm for method II) of maximum absorbance against the corresponding blank. Method III is based on the reaction of the drug with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of cerric ammonium sulphate, to form a colored species having λ max of 620 nm. All the variables have been optimized for the above three methods. The methods have been evaluated and are found to be precise and accurate. Recovery studies were carried out by standard addition method. These methods were extended to pharmaceutical formulations and there was no interference from any common pharmaceutical excipients and diluents.

Key words: Ofloxacin hydrochloride, Colorimetric methods, Erichrome black T,Orange G,3-methyl-2-benzothiazolinone hydrazone (MBTH).

Introduction

Ofloxacin[1] is chemically known as (±) -9-flouro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-di]-1,4-benzoxazine-6-carboxylic acid hydrochloride. (Figure.1).Ofloxacin belongs to Quinolone antibacterial category.Ofloxacin is used in the treatment of genito-urinary, respiratory, gastro-intestinal diseases, skin and soft tissue infection, peritonitis and gonorrhoea.

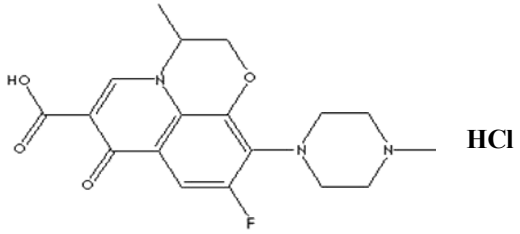


Figure 1: Structure of ofloxacin hydrochloride

Literature survey revealed that various extractive spectrophotometric, UV-derivative and HPLC methods have been reported. Spectrophotometric determination of ofloxacin hydochloride in pharmaceuticals and urine[2], with supracene violet 3B and tropaeolin 000[3], bromophenol blue and bromocresol purple[4], sulphonphthalein dyes[5], citric acid-acetic anhydride[6], bromothymol blue and bromophenol blue[7] and few RP-HPLC methods of ofloxacin hydrochloride alone or in combined dosage form[8-11]. The objective of this study is to develop accurate, precise, sensitive, selective and reproducible spectrophotometric methods for the determination of ofoxacin hydrochloride in bulk and its pharmaceutical dosage form.

Materials and methods

Instruments: All the measurements were made using Shimadzu UV-1800 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells. Drugs and chemicals were weighed by Metler balance

Chemicals and reagents: All the chemicals used were of analytical grade and procured from S.D.Fine Chemicals Ltd., Mumbai, India. Pharmaceutical grade ofloxacin hydrochloride was gifted by M/S Hetero drugs, Balanagar, Hyderabad, India, and certified to contain 99.99 % of ofloxacin hydrochloride. Both tablet 1 MEXAFLO (Sandoz) and Tablet 2 FLOSUN (Neosun Biotech) containing 200 mg of ofloxacin hydrochloride were procured from local drug store.

All the solutions were prepared with distilled water.

Erichrome black T (0.1 % w/v): 100 mg of EBT was weighed accurately and taken into 100 mL volumetric flask and dissolved with 30 mL of distilled water and diluted to mark with distilled water. The solution was treated with chloroform to remove any chloroform soluble impurities if present.

Orange G (0.2 % w/v): 200 mg of orange G was weighed accurately and transferred to 100 mL volumetric flask and dissolved by adding distilled water and diluted up to the mark. The solution is treated with chloroform to remove any chloroform soluble impurities if present.

Acetate Buffer (pH 3.5): 4 gm of anhydrous sodium acetate in about 840 mL of water and sufficient amount of glacial acetic acid to adjust pH to 3.5 (about 155 mL)

and diluted with water to 1000 mL.

Chloroform: Chloroform was purified as described by Vogel.

3-methyl-2-benzothiazolinone hydrazone (0.2 % w/v): 200 mg of MBTH was accurately weighed and dissolved in 100 mLof distilled water.

Cerric ammonium sulphate (1% w/v): 1 gm of cerric ammonium sulphate was dissolved in 20 mL of 0.1 N sulphuric acid and made up the volume to 100 mL.

Preparation of standard drug solution: 100 mg of ofloxacin hydrochloride was accurately weighed and dissolved in 100 mL distilled water in a standard volumetric flask to obtain a stock solution of 1 mg/mL. 10 mL of stock solution was diluted to 100 mL with distilled water (100 µg/mL).

Experimental

Method I and II:

Aliquots of the drug solution (0.25 -2.0 mL of 1 mg/mL for method I and 0.05-3.0 mL of 1 mg/mL for method II) were transferred into a series of 60 mL separating funnel. To each separating funnel, acetate buffer solution of pH 3.5 (2.5 mL for method I, 0.5 mL for method II) and dye solution (3 mL of 0.1% w/v erichorme black T solution for method I; 2.5 mL of 0.2 % orange G for method II) were added and mentioned in Table 1. The total volume of the aqueous phase was adjusted to 10 mL with distilled water. Then 10 mL of chloroform was added to each separating funnel. The contents were shaken for the thorough mixing of the two phases and were allowed to stand for clear separation of the layer. The absorbance of the separated chloroform layer was measured against their respective reagent blank at the wave length of 512 nm method I and 484 nm for method II.

Method III: Aliquots of standard solution (2.5- 25 µg/mL) of 1mg/mL were transferred into a series of 10 mL volumetric flask of each. Then 1.0 mL of MBTH and 1.0 mL cerric ammonium sulphate were added and the flasks were kept aside for 20 min. at room temperature. The solutions in each flask were made up to the mark with distilled water and the absorbance was measured at 620 nm against reagent blank. (Table 2)

Assay procedure: Twenty tablets were accurately weighed and an amount of tablet powder equivalent to 100 mg of ofloxacin hydrochloride was transferred into a 100 mL volumetric flask and the volume was made up to 100 mL with distilled water and filtered. Appropriate aliquots of drug solution were taken and the assay procedures were followed for analysis of drug contents. The results of analysis are given in Table 4.

Results and Discussions

Experiments were carried out to optimize the reaction condition for complete color formation. It was found that 3.0 mL of 0.1% EBT and 2.5 mL pH 3.5 acetate buffer solutions for method I and 2.5 mL of 0.2 % of orange G and 0.5 mL of acetate buffer solutions for method II were optimum for the achievement of maximum color intensity. For method III 1.0 mL of MBTH and 1.0 mL of cerric ammonium sulphate was found to be optimum to get the stable colour. The optical characteristic such as Beer's law limit, molar extinction co-efficient, correlation co-efficient (r), Sandell's sensitivity and % relative standard deviation (calculated from 6 measurement contains $3/4^{th}$ of the amount of the upper Beer's law limit of the method) were calculated for all three methods and the results are summarized in Table-3.Commercial formulation containing tablets were successfully analyzed by the proposed and reference methods. The values obtained by the proposed and reference methods for

formulation were compared statistically by the F and T test found that they did not differ significantly. As an additional demonstration of accuracy, recovery studies were performed by adding a fixed amount of the drug to the pre-analyzed formulations. The results are summarized in Table-4. The ingredients usually present in formulation of ofloxacin hydrochloride did not interfere with the proposed analytical methods.

Table 1: Optimum conditions and results of the proposed method for the determination of ofloxacin hydrochloride for method I and Method II

Reagent	Method I	Method II
Drug solution taken (µg/mL)	2.5--20	5--30
Volume of buffer (mL)	2.5	0.5
pH of buffer solution	3.5	3.5
Volume of reagent employed (mL)	3.0	2.5
λ max (nm)	512	484

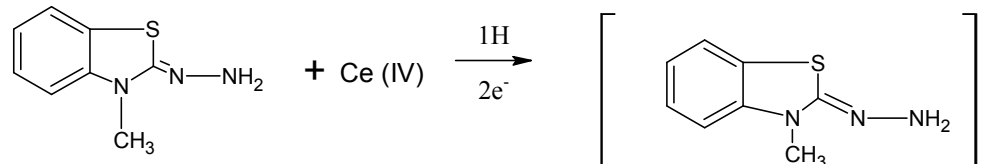
Table 2: Optimum conditions and results of the proposed method for the determination of ofloxacin hydrochloride For Method III

Reagent	Method III
Drug solution taken (µg/mL)	2.5-25
Volume of MBTH (mL)	1.0
Volume of CAS employed (mL)	1.0
λ max (nm)	620

Table 3: Regression characteristics of the proposed methods

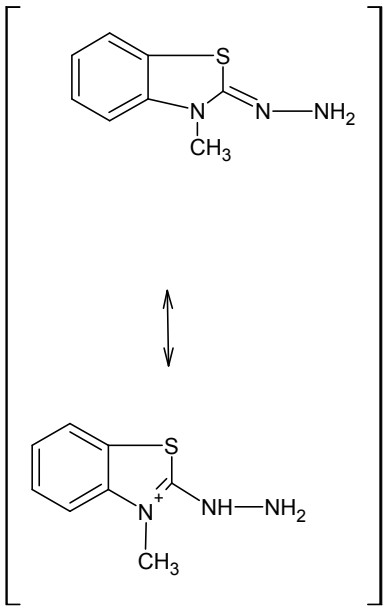
Parameters	Method		
	I	II	III
λ max nm	512	484	620
Beer's law limits(mcg/ml)	2.5--20	5--30	2.5-25
Sandell's sensitivity (mcg/cm^2/0.001 A.U)	0.04545	0.0360	0.02114
Molar Absorptivity (L mol^{-1}cm^{-1})	0.0954×10^6	0.1004×10^6	0.1720×10^6
Correlation coefficient(r^2)	0.999	0.999	0.999
Regression equation** (y=b+ac)			
Slope(a)	0.02182	0.02266	0.04488
Intercept(b)	0.02121	0.12530	0.04960
Range of errors*			
Confidence limit with 0.05 level	0.6164	0.1929	0.2344
Confidence limit with 0.01 level	0.9120	0.2854	0.3469
% Relative Standard Deviation*	0.7372	0.2308	0.2804

** y=b+ac
Where, Y is the absorbance and x is the concentration in µg/mL
*Average of eight determinations.

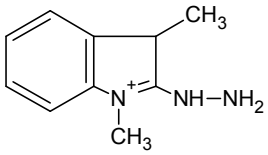


(2Z)-2-hydrazinylidene-3-methyl-2,3-dihydro-1,3-benzothiazole

(1)



Electrophilic Intermidiate (2)



one of the Intermidiate(3)

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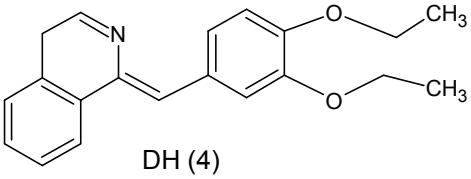
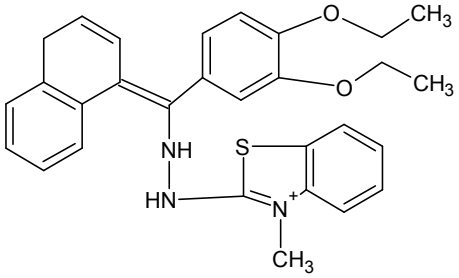


DH (4)



DH reagent complex(5)

Table 4: Assay and recovery o ofloxacin hydrochloride in pharmaceutical formulations

pharmaceutical formulations	Labeled amount found (mg)	Amount found in [a] (mg) using proposed methods ± S.D			Found by reference method [c] ±S.D	%Recovery by proposed methods [b] ± S.D		
		Method-1	Method-2	Method-3		Method-1	Method-2	Method-3
Tablet1	200	200.03 ±0.068 F=0.0005 T=0.1647	199.98 ±1699 F=0.0371 T=0.0371	199.99 ±0.1379 F=0.0081 T=0.1555	199.78 ± 0.4852	100.09 ± 0.0857	99.90 ± 0.216	99.91 ±0..2552
Tablet 2	200	200.09 ±0.120 F=0.0149 T=0.2391	199.79 ±0.4561 F=0.8861 T=0.7729	199.93 ±0.2248 F=0.1142 T=0.3578	199.68 ± 0.4879	100.03 ± 0.3091	99.84 ± 0.1562	99.97 ±0.1920

[a]Average ±standard deviation of eight determinations ,the F and T-values refer to comparison of proposed method with reference method .Theoretical values at 95% confidence limits T=2.365 and F= 4.88.

[b] Recovery of 10 mg added to the pre analyzed pharmaceutical formulations (average of three determinations).

[C] U.V method using 0.1M Hcl as solvent at λ max, 294nm**.**

Conclusion

In conclusion the proposed spectrophotometric methods for the estimation of ofloxacin hydrochloride are simple, sensitive, cheap and accurate and can be used for the routine quality control analysis of the drug in bulk and quantitative determination of oflaxacin hydrochloride from its pharmaceutical preparations.

Procedure for reference method (U.V.method):[9]

100 mg of ofloxacin hydrochloride was transferred to 100 ml volumetric flask and dissolved in 0.1 M hydrochloric acid and the volume was made up to 100 mL. The absorbance maxima and linearity was found to be 294 nm and 2-20 µg /mL.

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