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VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF GATIFLOXACIN

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ABSTRACT

Two new, accurate, precise, sensitive and reproducible visible spectrophotometric methods have been developed for the determination of Gatifloxacin in pure form and in tablet formulation. Method A is based on the oxidation of Gatifloxacin by a known excess amount of potassium chromate followed by the estimation of the unreacted amount of chromate by complexation with sym–diphenylcarbazide developing a violet color. The reacted oxidant corresponds to the Gatifloxacin content. At the maximum absorption of 548 nm, Beer's law is obeyed from 1 to10 mcg/ml of Gatifloxacin with a correlation coefficient r = 0.9988. Method B is an extractive spectrophotometric method based on the Mannich reaction of Gatifloxacin with m–nitro aniline and Para formaldehyde in acidic medium. The yellow base formed is extracted with chloroform and the maximum absorbance observed at 453 nm.The linearity range was 10–40 mcg/mL with a correlation coefficient r = 0.9999. The proposed methods were successfully applied for the analysis of marketed tablets and recovery studies were performed by adding 10 mg of pure Gatifloxacin to the pre analyzed sample. The results indicated that there is no interference from common additives.

Key words: Gatifloxacin, Potassium chromate, m–nitroaniline, Paraformaldehyde, Mannich base.

INTRODUCTION

Gatifloxacin[1] chemically is 1–cyclopropyl–6–fluoro–1,4–dihydro–8–methoxy–7–(3–methyl–1–piperazinyl)–4–oxo–3–quinolone carboxylic acid sesquihydrate.Gatifloxacin (GF) is fourth generation quinolone antimicrobial agent with broad spectrum of antimicrobial activity[2] and enhanced potency against respiratory pathogens. GF plays a useful role in treatment of acute exacerbations of chronic bronchitis, community acquired pneumonia, acute sinusitis and urinary tract infections[3,4]. GF is not official any pharmacopoeia. HPLC and UV spectrophotometric methods are available for the estimation of GF. This is the first report on visible spectrphotometric methods for the analysis of GF.

EXPERIMENTAL

Analytical grade reagents and double distilled water were used for the study. An ELICO UV–Visible spectrophotometer SL–159 was used for the spectrophotometric measurements.

Method A

GF stock solution was prepared by dissolving 0.1g of pure GF in 100 mL of water containing 10 mL of conc.HCl. A working standard of 100 mcg/mL was prepared by diluting 10 mL of stock solution to 100 mL with water in a standard flask.

Aliquots of working standard solution of GF from 0.1 to 1 mL were transferred to a series of 10 mL standard flasks and to each flask 5 mL of 5.2 mcg/mL of standard chromate solution (prepared by dissolving 0.373 g of pure potassium chromate in 1 L of 0.8 mol/L of HNO_3) was added. After mixing, 4 mL of reagent solution (Solution of 4.0 x 10^{-3} mol/L of sym–diphenyl carbazide in 250 mL of ethanol) was added and diluted to the mark with water. The absorbance was measured at 548 nm against a reagent blank and the readings were used for constructing the calibration curve (Fig.1).

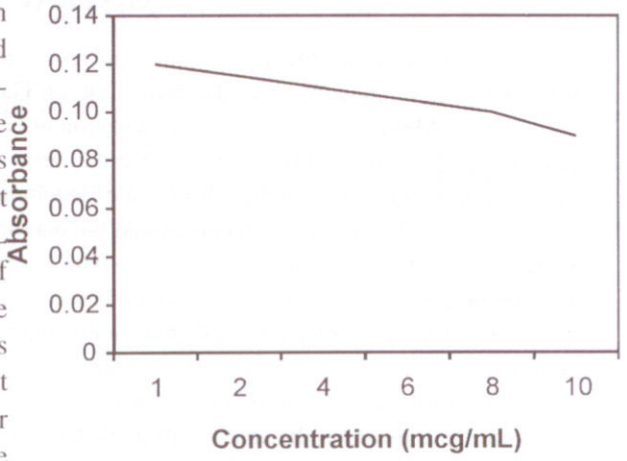

Fig. 1. Calibration Curve for Method A

Ten tablets were accurately weighed, finely powdered and powder equivalent to 0.1g of GF was dissolved in 100 mL of water, filtered, suitably diluted and the above procedure was adopted.

Method B

A standard stock solution of GF was prepared by dissolving 0.1 g of pure GF in 90 mL of ethanol and 10 mL of conc. HCl. Aliquots of standard solution of GF ranging from 1.0 to 4.0 mL was pipetted into series of 100 mL of graduated separating funnels. To each separating funnel, 60 mg of recrystallized m–nitroaniline (Chemspure), 20 mg of recrystallized paraformaldehyde (S.D.Fine) were added and shaken vigorously and set aside for 20 minutes. To this 20 mL of cold water was added. A yellow precipitate was formed, which was extracted successively with three quantities each of 50 mL, 25 mL and 25 mL of chloroform, respectively. The chloroform layers from the series of separating funnels were transferred to series of 100 mL standard flasks and the volume was made up to 100 mL with chloroform and the absorbance

was measured at 453 nm against a concomitantly prepared reagent blank. The readings obtained were used for constructing the calibration curve (Fig. 2).

Ten tablets were accurately weighed, powdered and powder equivalent to 0.1g of GF was dissolved in 10 mL of conc. HCl and 90 mL of ethanol, filtered, suitably diluted and the general procedure was adopted.

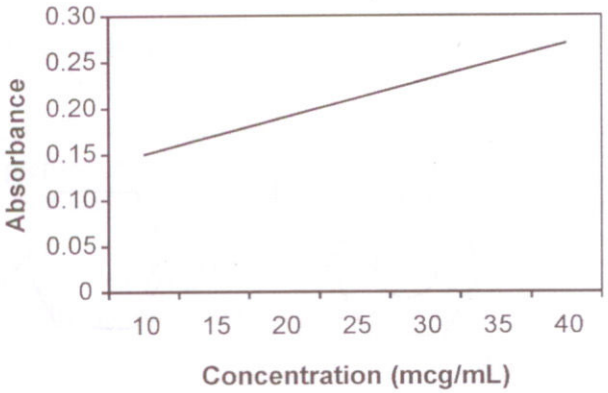

Fig. 2. Calibration Curve for Method B

Recovery studies were performed for both of the methods by adding 10 mg of pure GF to preanalysed samples and the measurements were repeated. Statistical validations of results were also done to evaluate the accuracy, precision and reproducibility.

RESULTS AND DISCUSSION

Method A involves oxidation of GF by a known excess of chromate in nitric acid medium and complexing the unreacted chromate with sym–biphenyl carbazide as chromate–diphenyl carbazide complex. GF, in increasing amounts, consumes chromate and decreases chromate–diphenyl carbazide complex absorbance. The absorbance is found to decrease linearly with increasing concentrations of GF which forms the basis for this determination. At acid concentration less than 0.05mol/L,the full color does not develop immediately and at acid concentrations above 0.8mol/L the complex is less stable[5,6]. The oxidized product of GF was isolated from the resultant mixture by means of TLC (Acetone : Chloroform–7 : 3, Silica GelGF_{254}, spot identified under UV light) and subjected to IR spectroscopy(IR : 2853 cm^{-1},1482 cm^{-1} $-CH_2$; 1450 cm^{-1} $-OCH_3$; 3040 cm^{-1} cyclopropane; 1560 cm^{-1} –NH; 1220 cm^{-1} –C–N, tertiary; 1715 cm^{-1} –C = O, 6 membered; 3500 cm^{-1} –COOH;1010 cm^{-1} C–F; 1640 cm^{-1}–diketone; 1682 cm^{-1}–lactam) The IR data indicate that GF is oxidized by the reagent at the double bond between carbon atoms 2 and 3 yielding a diketone. The scheme is as follows:

$$2\ C_{20}H_{22}N_3O_4F + 2\ CrO_4^{2-} + 12\ H^+ \rightarrow 2\ Cr^{3+} + 2\ C_{20}H_{22}N_3O_5F + 6\ H_2O$$

Unreacted CrO_4^{2-} + sym–DPC → Cr–DPC complex.

Method B involves the Mannich reaction of GF with m–nitroaniline and paraformaldehyde in acidic medium[7]. The IR spectrum of the yellow precipitate was in accordance with the structure of the Mannich base (Fig 3). (IR: 2853 cm^{-1}, 1482 cm^{-1} $-CH_2$; 1450 cm^{-1} $-OCH_3$; 3040 cm^{-1} cyclopropane; 1560 cm^{-1} –NH; 1220 cm^{-1} –C– N, tertiary; 1715 cm^{-1} –C = O, 6 membered; 3500 cm^{-1} –COOH; 1010 cm^{-1} C–F; 1325 cm^{-1} $-C-NO_2$, aromatic).

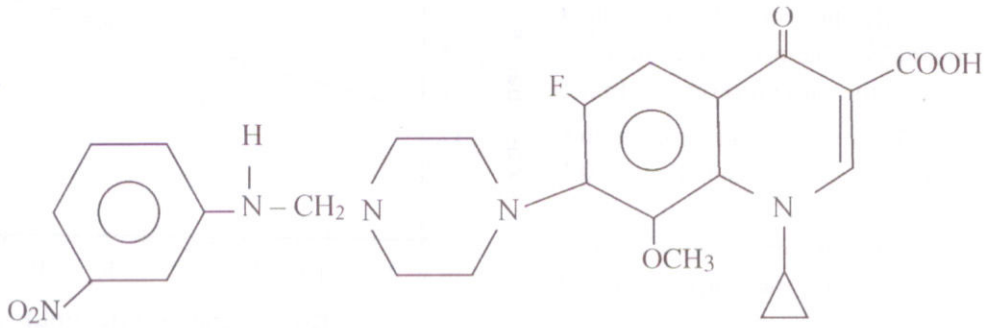

Fig. 3. Structure of Mannich base

The yellow chromogen has maximum absorbance at 453 nm.Optical characteristics for the proposed methods are reported in Table 1. The results of analysis of marketed tablets of GF by the proposed methods are reported in Table 2. The color produced in Method A was found to be stable for more than 48 hrs. The color produced in Method B was found to be stable for 45 to 60 minutes. Satisfactory results from recovery studies indicated that none of the common additives and excipients interfere the assay methods. The proposed methods were accurate, precise and reproducible as indicated by the statistical values. Method A is more simple, sensitive and rapid method which may be used for the routine analysis of Gatifloxacin tablets.

Table 1. Optical characteristics of proposed methods

Parameters	Method A	Method B
λ_{max} (nm)	548	453
Beer's law limit(mcg/mL)	1–10	10–40
Molar absorptivity(mol/Lit/cm)	$9,276 \times 10^2$	6.678×10^3
Sandell's sensitivity	15×10^{-3}	25×10^{-3}
Correlation coefficient(r)	0.9988	0.9999
Regression equation(Y=absorbance and x = Concentration)	Y = 0.007x – 0.0026	Y = 0.0168x +0.001
Standard error of correlation coefficient	0.0046	0.0083
% Relative standard deviation	0.228%	0.40%
99% Confidential limits	0.114–0.185%	0.152–0.347%
Limit of detection mcg/mL	0.05	10

Table 2. Analysis of marketed tablets of Gatifloxacin

Formulation	Labeled amount mg	Amount found by proposed methods				% Recovery	
		Method A		Method B		Method A	Method B
		mg	%	mg	%		
Gaity tablets	200	199.96	99.96	199.98	99.99	99.43	101.66
Gatilox tablets	200	202.8	101.04	2010.6	100.53	99.06	99.40

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