SPECTROPHOTHOMETRIC DETERMINATION OF GATIFLOXACIN THROUGH COMPLEXATION WITH SURFACTANT

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Abstract

A simple, quick and sensitive U.V spectrometric method has been developed for the quantitative estimation of Gatifloxacin in bulk and pharmaceutical formulation. The method was based on the complexation with neutral surfactant, Tween-20 in aqueous phosphate buffer at PH 7.4 which showed an absorption maximum at 295nm and obeyed Beer's law in the concentration range 2µg/ml to 14µg/ml which was based on the formulation of a U.V sensitive complex known as Europium. The limit of detection and quantification were calculated and relative standard deviation were less than 1.242. The results of analysis for the method has been validated statistically and by recovery studies the results obtained with the proposed method were in agreement with the labeled amounts.

Key words : Gatifloxacin, Spectrophotometer, Tween-20.

1. Introduction

Gatifloxacin is chemically 1-cyclopropyl-6-fluoro- 8-methoxy-7-(3-methylpiperazin-1-yl) - 4-oxo-quinoline-3-carboxylic acid. The flouroquinolones enter the bacterium by passive diffusion through water filled protein channels (Porins) in the outer membrane. Once inside the cell, they inhibit the replication of bacterial DNA by interfering with the action of DNA gyrase (topoisomeraseII & topoisomeraseIV) during bacterial growth & reproduction. Binding of quinolone to both enzyme& DNA forms a ternary complex that inhibits the resealing step, & can cause cell death by inducing cleavage of DNA. Because DNA gyrase is a bacteriospecific target for antimicrobial therapy, cross-resistance with other, more commonly used antimicrobial drug is rare but this is increasing in the case of multi drug resistant organisms. The second site blocked by the flouroquinolones – topoisomeraseIV is required by bacteria for cell division. It has been implicated in the process of segregating newly replicated DNA in gram negative organisms (Escherichia coli), the inhibition of DNA gyrase is more significant than that of topoisomeraseIV, whereas in Gram-Positive organisms (Staphylococci)[1-2].

The literature survey reveals that Gatifloxacin has been estimated in plasma by non aqueous titration[3] by using Perchloric acid, Chromatography[4-13], spectrofluorometric[14], capillaryelectrochromatography[15], electrochemical[16] and spectrophotometric[17] methods have been reported. No Spectrophotometric methods in aqueous media are cited in the literature. We report a simple and sensitive micelle based spectrometric sensitive complex using surfactants in aqueous buffer for the analysis of Gatifloxacin.

2. Experimental Procedure

2.1 Chemicals and Instrumentation

The study was carried out by using PERKIN ELMER U.V Spectrophotometer equipped with 10mm matched Quartz cells. All reagents and chemicals used were of Analytical grade. Hydrochloric acid, Potassium di hydrogen phosphate, sodium hydroxide, Tween-20 , Tween -80 and distilled water. Tablet formulation (Gaity - 200 & 400 Dr Reddys Labs, Hyderabad) procured from local market.

2.2. Selection of Solvents:

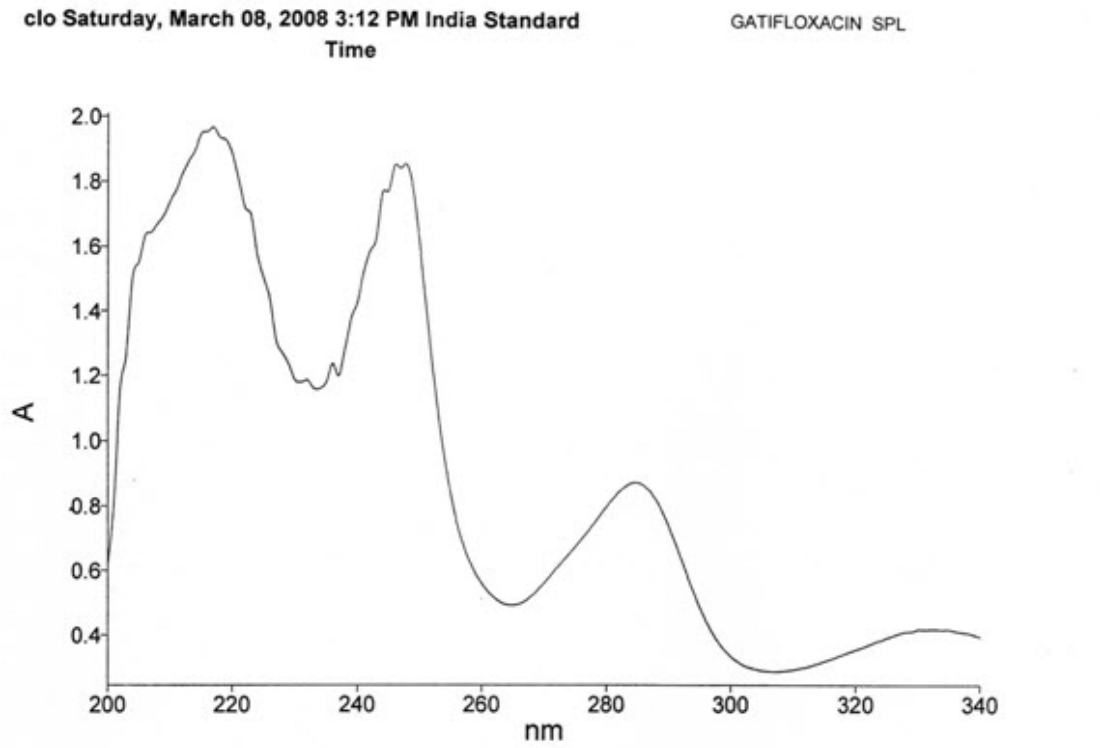
The solvent was selected by determining the solubility of Gatifloxacin in various solvents such as 0.1 N sodium hydroxide,0.1N Hydrochloric acid ,P^H: 3.2 Acid buffer, P^H:4.0 Acid buffer, P^H: 5.8 Phosphate buffer, P^H: 6.8 Phosphate buffer, P^H:7.4 Phosphate buffer, P^H 7.4 phosphate buffer +Tween20(0.1%) and P^H7.4 Phosphate buffer+Tween 80(0.1%)

Table No:1 Effect of solvent on absorption intensity of Gatifloxacin in presence of Tween20 and Tween 80.

Solvent	**Tween20**	**Tween80**
0.1 Hydrochloric acid	0.13	0.07
0.1n Sodium hydroxide	0.07	0.01
Phosphate buffer P^H3.2	0.09	0.02
Phosphate buffer P^H4.0	0.13	0.08
Phosphate buffer P^H5.2	0.16	0.11
Phosphate buffer P^H6.8	0.23	0.22
Phosphate buffer P^H7.4	**0.8**	**0.41**
Phosphate buffer P^H9.0	0.54	0.28
Phosphate buffer P_H10.0	0.5	0.2

2.3 COMPLEXATION OF GATIFLOXACIN WITH SURFACTANT

An accurately weighed quantity about 100mg Gatifloxacin was taken in 100ml volumetric flask and was dissolved in P^H 7.4 Phosphate buffer containing 0.1% Tween 20 and volume made up to the mark. To get the concentration of 1mg/ml , the aliquots portion of stock solution of Gatifloxacin was diluted with P^H7.4 Phosphate buffer containing 0,1% Tween20 to obtain a concentration of 10µg/ml . Measure the absorbance of the complex by scanning between 200nm to 400nm against a blank. The λ max of the complex was obtained at 295nm.



Sample ID	GATIFLOXACIN SPL.Sample	
Description	L'AMDA MAX DETERMINATION OF GATIFLOXACIN SPL	
Threshold	0.100	
Range start (nm)	340	
Range end (nm)	200	
Find	Peaks	
Display	List by position	

Fig. 1 U.V.spectrum of Gatifloxacin-Tween20 complex

2.4 Analysis of formulation

An accurately weighed amount of freshly powdered tablet equivalent to 100mg of the drug was dissolved in a 20ml of P^H 7.4 Phosphate buffer containing 0.1% Tween-20 and after 15minutes of mechanicsal stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper and necessary amount of filtrate was added and diluted to required concentrations and the absorbance was measured at 295nm.

3 Optimization of Reaction Conditions

3.1 Effect of P^H on Surfactant :

At acidic P^H there was formation of turbidity and at basic P^H the solubility of Gatifloxacin was poor as it was in aqueous media, so the solubility was enhanced by using surfactants such as anionic surfactants and neutral surfactants. Gatifloxacin was cationic drug and formed a fluorescent complex with an anionic surfactant. Gatifloxacin showed complete solubility with a neutral surfactant such as Tween-20 and Tween-80 but Tween-20 gave a very clear solution which is highly U.V sensitive than Tween-80.At the P^H 7.4 phosphate buffer containing 0.1% tween-20 gave max absorbance value.

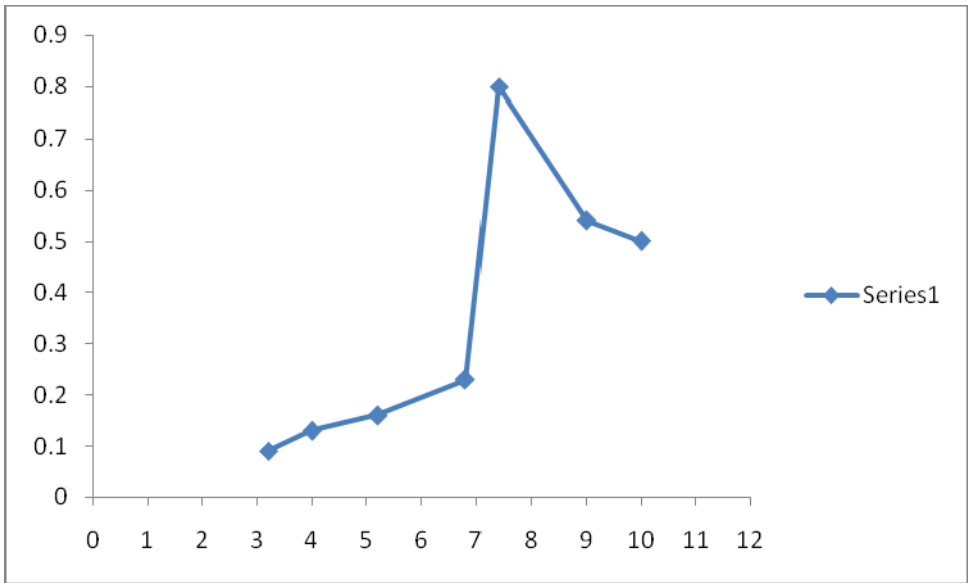
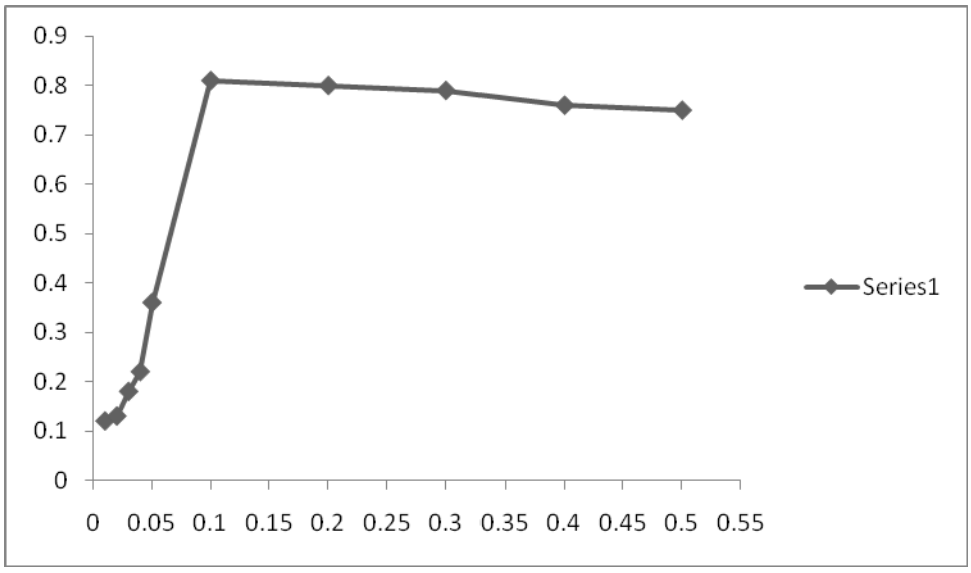


Fig No:2 Effect of P^H on absorption intensity of gatifloxacin-tween20 complex (x-axis- P^H of Phosphate buffer, y-axis-Absorbance)

3.2 Effect of Concentration on Surfactant

To 1ml of the Gatifloxacin stock solution , aliquot portion of 0.01% to 0.05% of Tween -20 in P^H 7.4 phosphate buffer is added and make up the volume to 100ml and absorbance values at 295nm were taken. Investigation of the surfactant concentration revealed that 0.1% to 0.5% of Tween-20 was found to be optimum for maximum complexation of Gatifloxacin using 100µg/ml concentration.



FigNo:3 Effect of Concentration of surfactant on the absorbance intensity of Gatifloxacin –Tween20 complex (x-axis Concentration of surfactant, y-axis Absorbance)

3.3 Linearity Range and Quantification Procedure

Beers law was found to be obeyed in the concentration range 2µg to 14 µg/ ml at 295 nm .A(1%,1c.m) equal to $8.23x10^2$. L mole-1cm-1.The drug surfactant complex absorbances were fitted to the equation Y=a+bx, where Y is the absorbance at relevant maximum ,b is the slope and a is the intercept of the calibration curve. The correlation coefficient was found to be 0.996 indicating exact linearity. The accuracy of the proposed procedure were 100.3 and 100.4. Repeatability and reproducibility were evaluated and RSD% ranged from 0.106 to 1.242.

Table No:2 Statistical analysis of calibration graph and analytical data for complexation of Gatifloxacin with tween 20 in Phosphate buffer P^{H}7.4

parameters	Results
wavelengthλmax	295
Beer'slaw limit µg/ml	2-14
molarabsorptivity€ $Lmol^{-1}cm^{-1}$	$8.23x10^{-2}$
Regression equation	
a)slope(a)	0.065
bIntercept(b)	0.017
correlation coefficient(r)	0.996
Detection limit µg/ml	1.17
Quantification limit µg/ml	3.10
RSD%	1.519-1.591

3.4 Effect of Common Excipients on Analysis of Gatifloxacin

Gatifloxacin was mixed with necessary amounts of common excipients and dissolved in a 20ml of PH 7.4 Phosphate buffer containing 0.1% Tween20 and after 15minutes of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper and necessary amount of filtrate was added and diluted to 100ml and then the same procedure followed as above.

Table no:3 Analysis of Gatifloxacin in presence of common excipients using the proposed method

Ingredient	Recovery ±S.D%
Glucose	99.8±0.054
Lactose	100.3±0.85
Magnesium state	100.1±0.22
Starch	99.3±0.59

3.5 Assay of Dosage Form:

An accurately weighed amount of freshly powdered tablets equivalent to 100mg of the drug was dissolved in a 20ml of PH 7.4 Phosphate buffer containing 0.1% Tween20 and after 15minutes of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper and necessary amount of filtrate was added and diluted to 100ml and then the same procedure followed as above**.**

Table No:4 Determination of Gatifloxacin in pharmaceutical dosage forms

Sample	Recovery±S.D% Proposed method	Recovery±S.D% Reference method
Gaity200	100.3±0.1242	99.6±0.73
Gaity400	100.4±0.1068	99.6±0.73

3.6 Results and Discussion:

The Linearity range of gatifloxacin –tween -20 comlex at pH7.4 covered over a range of 2-14µg/ml o the drug with A(1%1cm)equals to $8.23x10^{2}Lmol^{-1}cm^{-1}$.The drug surfactant complex absorbances were fitted to the equation Y=a+bx, where Y is the absorbance at relevant maximum ,b is the slope and a is the intercept of the calibration curve. The correlation coefficient was found to be 0.996 indicating exact linearity. The accuracy of the proposed procedure were 100.3 and 100.4. Repeatability and reproducibility were evaluated and RSD% ranged from 1.06 to1.242.

3.7 Conclusion

The proposed procedure is stability indicating aqueous method one which can be used for the determination without interference in dosage form. The drug being poorly water soluble, the solubility has been enhanced by complexation with surfactant and considered more selective in determining the intact drug. In addition ,there is background interference of the complex matrix and the method resolved the individual drug, drug additives and drug decomposition both interfered.

4. References:

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