Spectrophotometric Determination of Gatifloxacin in Pharmaceutical Formulations and Biological Samples

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Three new simple and sensitive spectrophotometric methods in ultraviolet region have been developed for the determination of gatifloxacin in bulk drug, pharmaceutical preparations and biological samples. Gatifloxacin exhibited maximum absorbance at 289 nm (method A) with apparent molar absorptivity of 1.23×10^4 l/mol×cm when dissolved in sodium hydroxide; and maximum absorbance at 292 nm (method B) with apparent molar

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absorptivity of 1.71×10^4 l/mol×cm when dissolved in hydrochloric acid. Third developed method (method C) was based on the formation of yellow coloured chromogen with ferric chloride and potassium dichromate, which showed maximum absorbance at 352 nm with apparent molar absorptivity of 1.23×10^4 l/mol×cm. Beer's law was obeyed in the concentration range of 5-30 µg/ml. Results of analysis of all methods were validated statistically and by recovery studies.

Gatifloxacin (GFN), 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, is an advanced generation antibiotic[1]. It is used in the treatment of susceptible infections, including respiratory and urinary tract infections. It is official in Martindale's complete drug reference[2]. A survey of literature revealed a few high performance liquid chromatographic methods for its determination in human plasma using UV[3,4] and tandem mass detection[5]. No spectrophotometric method has been so far reported. Hence an attempt was made to develop simple and economical spectrophotometric methods for the analysis of GFN in pharmaceutical preparations and biological samples.

This paper describes three simple spectrophotometric methods for GFN, using 0.1M hydrochloric acid (method A), 0.1M sodium hydroxide (method B), and ferric chloride and potassium dichromate for the third method. In the first method, GFN exhibits the maximum absorbance at 289 nm, and in the second method it gives maximum absorbance at 292 nm. In the third method, GFN reduces ferric chloride to ferrous, which forms complex with potassium dichromate to yield a yellow coloured chromogen having maximum absorbance at 352 nm.

All the chemicals used were of analytical grade. Aqueous solutions of hydrochloric acid (0.1 M), sodium hydroxide (0.1 M), ferric chloride (0.5%) and potassium dichromate (0.5%) (Merck India Limited, Mumbai) were prepared in distilled water. Spectral and absorbance measurements were made on Shimadzu 1601 UV/Vis spectrophotometer with 1 cm matched quartz cells.

Working standard solutions (Hetero Drugs Limited, Chennai) were prepared by dissolving 100 mg of GFN in 100 ml of 0.1 M hydrochloric acid and in 100 ml of 0.1 M sodium hydroxide separately for methods A and B; and by dissolving 100 mg of GFN in 2-3 ml of 0.1 M hydrochloric acid and then diluting to the mark with distilled water in a 100 ml standard flask for method C. Aliquots of stock solutions were then diluted with the respective diluents separately to get final concentrations of 5, 10, 15, 20, 25 and 30 µg/ml. The absorbances were then measured at 289 nm against 0.1 M sodium hydroxide, and at 292 nm against 0.1 M hydrochloric acid. To aliquots of stock solution, 1 ml of ferric chloride and 2 ml of potassium dichromate were added and the volume was made up to 100 ml with distilled water. The solution was allowed for 2-3 min. The absorbance of the developed yellow-coloured chromogen was measured at 352 nm.

For the analysis of GFN in formulations, three different preparations 200 mg (Nicholas Piramal), 400 mg (Sarabhai Piramal) and IV of 10 mg/ml strength (Nicholas Piramal) were taken. Twenty tablets were weighed and powdered. The tablet powder equivalent to 100 mg of GFN each were weighed accurately and dissolved in 0.1 M hydrochloric acid and 0.1 M sodium hydroxide separately. The solutions were diluted to 100 ml using the respective solvents and filtered through Whatman filter paper No. 40. One ml of IV (10 mg/ml) solution was diluted to 10 ml with distilled water. Appropriate aliquots of solutions were taken and analyzed for GFN, using all the three procedures described earlier.

A known amount of GFN was added to 5 ml of urine sample. To this was added 0.5 g of lead nitrate to precipitate out the chlorides present. The solution was filtered and the excess of lead present in the filtrate was removed by adding 8 M sulphuric acid. The solution was again filtered. A suitable amount of an aliquot was analyzed by method C for the quantification of GFN as described for pure drug.

One millilitre of blood was spiked with a known amount of GFN before the addition of sodium citrate. The citrated blood was deproteinated with trichloroacetic acid and filtered. The filtrate was diluted with distilled water to 100 ml in a standard flask. An appropriate amount of an aliquot was taken, neutralized with dilute sodium hydroxide solution and analyzed by method C as described earlier.

Synthetic mixture containing talc, starch, sucrose, lactose, gelatin and magnesium stearate (50 mg each) and 50 mg of GFN was prepared, and a portion of the mixture

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA

Parameters	Method A	Method B	Method C
λ_{max}	289 nm	292 nm	352 nm
Beer's law limit (μg/ml)	5 to 30	5 to 30	5 to 30
Molar absorptivity (l/mol×cm)	1.23×10^4	1.71×10^4	1.23×10^4
Correlation coefficient	1.004	0.997	1.003
Sandell's sensitivity (μg/cm² absorbance unit/0.01)	0.305	0.219	0.305
Regression equation (Y=bx+a)[a]			
Slope (b)	0.0667±0.036	0.0851±0.164	0.02001±0.042
Intercept (a)	0.024	0.051	0.045
Relative Standard deviation(n=6), %	0.64	0.20	0.32

a – with respect to Y = bx + a, where 'x' is the concentration in μg/ml

containing known amount of GFN was weighed accurately. The drug was extracted with 0.1 M sodium hydroxide and filtered, and the residue was washed five times with 0.1 M sodium hydroxide. The filtrate and the washings were then combined in a 100 ml calibrated flask and diluted up to the mark with distilled water, and the amount of GFN was determined by method C as described earlier.

The optical characteristics like molar absorptivity, Sandell's sensitivity and the linear regression equation of the above-mentioned methods are shown in Table 1. GFN exhibited maximum absorbance at 289 nm in 0.1 M sodium hydroxide, 292 nm in 0.1 M hydrochloric acid and at 352 nm by forming a complex with ferric chloride and potassium dichromate. A linear correlation was found between absorbances and concentration of GFN in the range of 530 μg/ml. The results of analysis and recovery studies are presented in Table 2. The percentage recovery values close to 100% indicate that there is no interference of the excipients present in the formulation.

TABLE 3: ANALYSIS OF GFN IN URINE AND BLOOD SAMPLES

Sample	GFN present, μg/ml	GFN found *μg/ml	Recovery ±SD, %
Blood 1	5.0	4.92	99.96±0.45
Blood 2	10.0	10.01	99.9±0.47
Blood 3	15.0	14.92	100.4±0.021
Urine 1	5.0	4.98	99.94±0.26
Urine 2	10.0	9.97	99.98±0.85
Urine 3	15.0	14.98	99.68±0.72

* - Average of five determinations, GFN = Gatifloxacin

The extent of interference by commonly associated excipients such as magnesium stearate, starch, talc, gelatin, dextrose, lactose and sucrose was determined by measuring the absorbance of a solution containing 10 μg/ml of GFN. An error of ±2% in the absorbance readings was considered tolerable. The proposed method was found to be free from interferences by the excipients in the levels found in dosage forms. This was quite clear from the data obtained on the analysis of synthetic mixtures. The results of analysis of urine and blood samples are shown in Table 3. As no spectrophotometric method for analysis of GFN in pharmaceutical and biological samples is currently available, the data obtained by the proposed method could not be compared for its validation. However, the data of analysis was supported by RSD values. Hence the developed methods were found to be sensitive, accurate, precise, repeatable and reproducible and can be used for the routine analysis of GFN in bulk drug, formulations and biological samples.

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TABLE 2: ANALYSIS OF GATIFLOXACIN FORMULATIONS

Formulation	Label claim (mg)	Amount found (mg)	% label claim* ± Standard deviation	Standard error	% recovery*
Method A					
Brand 1	200	200.3	100.1±0.48	0.247	100.4
Brand 2	400	401.3	100.3±0.62	0.680	99.9
Brand 3	10	10.09	100.9±0.011	0.124	101.8
Method B					
Brand 1	200	199.96	99.98±0.20	0.105	99.6
Brand 2	400	402.4	100.6±0.81	0.212	98.7
Brand 3	10	10.02	100.2±0.016	0.176	99.6
Method C					
Brand 1	200	200.1	100.02±0.27	0.127	100.1
Brand 2	400	400.68	99.96±0.67	0.519	99.98
Brand 3	10	10.01	99.98±0.72	0.312	99.92

1 - GRES and 3 - GATRIM *- (IV Concentrate injection) - -Nicholas Piramal India Ltd., Mumbai; 2 – GABACT- Sarabhai Piramal Pharmaceuticals Ltd., Vadodara.
* - Results of five replicates

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