Optimized and Validated Spectrophotometric Methods for the Determination of Gatifloxacin in Pharmaceuticals using N-Bromosuccinimide and Three Dyes

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Three new visible spectrophotometric methods which are simple, sensitive and cost-effective are proposed for the determination of gatifloxacin (GTF) using N-bromosuccinimide (NBS), and three dyes, thymol blue, indigo carmine and methyl orange as reagents. The methods engross the addition of a known excess of NBS to GTF in hydrochloric acid medium followed by determination of unreacted oxidant after a preset time by reacting with a fixed amount of either thymol blue and measuring the absorbance at 550 nm (Method A) or indigo carmine and measuring the absorbance at 610 nm (Method B) or methyl orange and measuring the absorbance at 520 nm (Method C). In all the methods, the amount of NBS reacted corresponds to the amount of GTF and the absorbance is found to increase linearly with concentration of GTF. Under the optimum conditions, GTF could be assayed in the concentration range 0.5-10, 0.5-7.5 and 0.5-1.5 $\mu g\ mL^{-1}$ by method A, method B and method C, respectively. The apparent molar absorptivities are calculated to be 3.4×10^4, 2.9×10^4 and $1.8 \times 10^5\ L\ mol^{-1}cm^{-1}$ for method A, method B, and method C, respectively, and the corresponding Sandell sensitivity values are 0.012, 0.014 and 0.002 $\mu g\ cm^{-2}$. The limits of detection and quantification are also reported. The intra-day and inter-day precision and accuracy of the methods were evaluated as per the current ICH guidelines. The methods were successfully applied to the determination of GTF in pharmaceutical preparations without interference from any of the pharmaceutical adjuvants. The results are in agreement with the reference method. The reliability of the methods was further checked by recovery experiments.

Key Words: Gatifloxacin, Assay, Spectrophotometry, N-bromosuccinimide, Formulations

Introduction

Gatifloxacin (GTF) is a synthetically derived, broad spectrum fluoroquinolone designed for both oral and intravenous administration. Chemically, it is ($\pm$)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesqui hydrate [1] (Fig.1). It is indicated for acute pyelonephritis, acute bacterial exacerbation of chronic bronchitis and complicated UTI.

Various techniques have been used for the determination of GTF in body fluids and pharmaceuticals. High performance liquid chromatography (HPLC) has been applied for the determination of the drug in plasma [2,3], serum [4], and serum and urine [5]. The drug in urine and serum has also been quantitated by spectrofluorimetry [6]. There is only one report on the application of HPLC for the assay of GTF in bulk and dosage forms. A non-aqueous titration procedure [7] has recently been described for the assay of drug in pharmaceutical formulations using perchloric acid as titrant. Very recently, Salgado *et al*.[8] have reported a microbiological assay for GTF in pharmaceutical formulations. Several UV-spectrophotometric [9-13] procedures employing different media have also been reported for assay in single as well as combined dosage forms.

Visible spectrophotometry, because of simplicity and cost effectiveness, sensitivity and selectivity, and fair accuracy and precision has remained competitive in an era chromatographic techniques for pharmaceutical analysis. Many visible spectrophotometric methods based on different reaction schemes are found in the literature for the assay of GTF. In a method reported by Dhachinamoorthy *et al*. [14] ferric ferricyanide was reduced by GTF and the blue chromogen formed was measured forming the basis of assay. A yellow-orange chromogen formed when GTF was treated with cerium(IV) was used by Devala and Babu [15] for the determination of drug in 40-160 $\mu g\ mL^{-1}$ range in dosage forms. Two methods, one based on redox-complexation reactions involving chromium (VI) and sym-diphenylcarbazide and the other on Mannich reaction, have recently been reported by Saraswathi *et al*. [16]
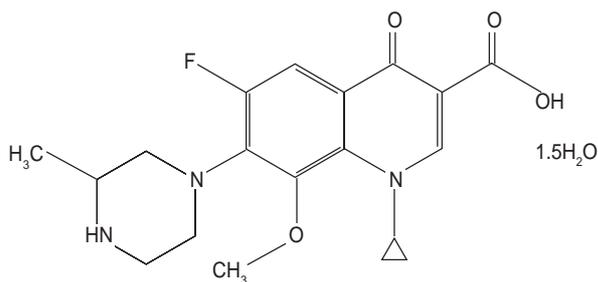

Fig. 1: Structure of gatifloxacin sesqui hydrate

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Three sensitive methods [17] based on chloroform extractable ion-association complexes formed by GTF with wool fast blue BL, Tropaeolinooo or bromophenol blue are also found in the literature. The reported visible spectrophotometric methods, although a couple of them sensitive, suffer from one or the other disadvantage such as poor sensitivity [14-16] or liquid-liquid extraction step [16,17].

The present investigation aims to develop sensitive and cost-effective methods for the determination of GTF in pure form and in dosage forms using visible spectrophotometry. The methods utilize NBS as oxidimetric reagent, which has successfully been used for the sensitive spectrophotometric determination of many bioactive substances [18-26]. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and Standards

All chemicals used were of analytical purity grade and all solutions were prepared in distilled water.

N-bromosuccinimide (NBS): An approximately 0.01 M NBS solution was prepared by dissolving about 0.2 g of chemical (SRL Research Chemicals, India) in water with the aid of heat and diluted to 100 mL with water and standardized [27]. It was diluted appropriately to get 320, 340 and 80 mg μL^{-1} NBS for use in method A, method B and method C, respectively. The NBS solution was stored in a refrigerator when not in use.

Hydrochloric acid (5M): Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5 M acid for method A and subsequently diluted to 2M and 1M for method B and method C, respectively.

Thymol blue (200 μg mL^{-1}): A 1000 μg mL^{-1} dye (Lobachem, Mumbai, India) solution was first prepared by dissolving accurately weighed 100 mg of dye in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. The stock solution was diluted five-fold to obtain the working concentration.

Indigo carmine (200 μg mL^{-1}): A 1000 μg mL^{-1} stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (S.d. Fine Chem., Mumbai, India, 90% dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution

was then diluted 5-fold to get the working concentration of 200 μg mL^{-1}.

Methyl orange (50 μg mL^{-1}): To prepare 50 μg mL^{-1} methyl orange, first, a 500 μg mL^{-1} dye solution was first prepared by dissolving accurately weighed 58.8 mg of dye (S.d. Fine Chem., Mumbai, India, 85% dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was further diluted to obtain a working concentration.

Standard solution of Gatifloxacin: Pharmaceutical grade GTF, certified to be 99.85 % pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A 1000 μg mL^{-1} solution of GTF was prepared by dissolving accurately weighed 100 mg of pure drug in 25 mL water with the aid of heat to get clear solution, followed by diluting to 100 mL with water in a calibrated flask. This stock solution (1000 μg mL^{-1}) was diluted with water to get working concentrations of 25, 25 and 5 μg mL^{-1} GTF for method A, method B and method C, respectively.

Methods

Method using Thymol blue (Method A)

Aliquots of pure GTF solution (0.5 to 4.0 mL; 25 μg mL^{-1}) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 4.0 mL with water. To each flask were added 1mL of 5M hydrochloric acid followed by 1mL of NBS (320 μg mL^{-1}). The content was mixed well and the flasks were set aside for 5 min with occasional shaking. Finally, 1 mL of 200 μg mL^{-1} thymol blue solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 550 nm against reagent blank after 10 min.

Method using indigo carmine (Method B)

Varying aliquots (0.5-3.0 mL) of standard 25 μg mL^{-1} GTF solution were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 3.0mL with water. To each flask were added 1mL each of 2 M hydrochloric acid and 1.0mL of 340 μg mL^{-1} NBS by means of micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 200 μg mL^{-1} indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against a reagent blank after 10 min.

Method using methyl orange (Method C)

Varying aliquots (0.5-3.0mL) of standard 5 μg mL^{-1} GTF solution were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 3.0mL with water. To each flask were added 1mL each of 1 M hydrochloric acid and 1.0mL of

80 µg mL^{-1} NBS by means of micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 50 µg mL^{-1} methyl orange solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 10 min.

In all methods, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Assay procedure for formulations

An amount of finely ground tablet powder equivalent to 100 mg of GTF was accurately weighed into a beaker, 50 mL water was added and stirred for 20 min and warmed. Then, the volume was made up 100 mL calibrated flask with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL^{-1} GTF) was diluted appropriately to get required concentrations for analysis by the proposed methods.

Results and Discussion

Optimization of experimental conditions

The proposed spectrophotometric methods are indirect and are based on the determination of the residual NBS after allowing the reaction between GTF and a measured amount of NBS to be complete. The surplus oxidant was determined by reacting it with a fixed amount of either thymol blue, indigo carmine or methyl orange dye. The methods make use of bleaching action of NBS on the dyes, the decolouration being caused by the oxidative destruction of the dyes.

GTF when added in increasing amounts to a fixed amount of NBS consumes the latter proportionately and there occurs a concomitant fall in the amount of NBS. When a fixed amount of dye is added to decreasing amounts of NBS, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of GTF (Figs. 2, 3 and 4).

Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these was found to be 20 µg mL^{-1} for both thymol blue and indigo carmine and 5 µg mL^{-1} for methyl orange. A NBS concentration of 32 µg mL^{-1} was found to irreversibly destroy the blue colour of 20 µg mL^{-1} thymol blue whereas 34 µg mL^{-1} NBS was required to bleach blue colour due to 20 µg mL^{-1} indigo carmine and a NBS concentration of 8 µg mL^{-1} was found to irreversibly destroy the red colour of 5 µg mL^{-1} methyl orange. Hence, different amounts of GTF were reacted

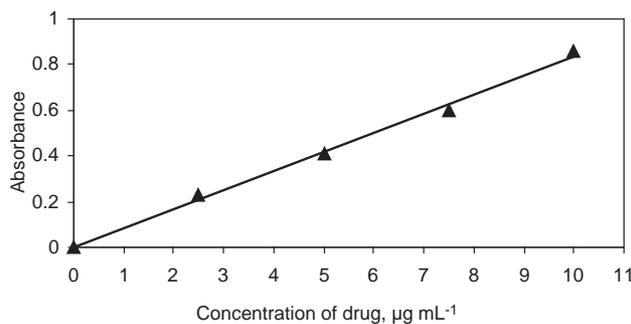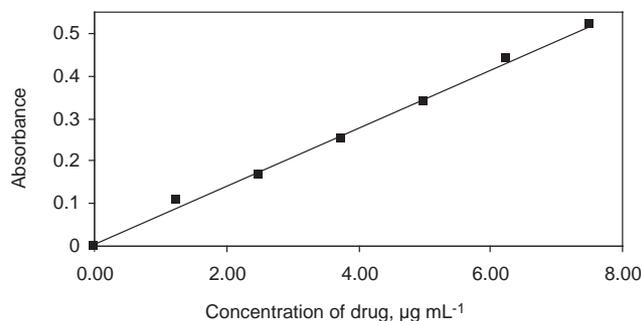*Fig. 2: Beer's law curve for method A*
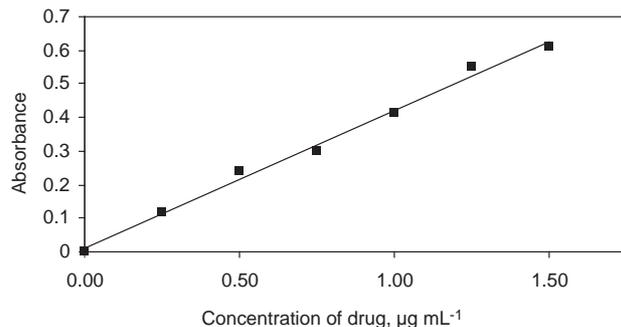

Fig.3: Beer's law curve for method B

Fig. 4: Beer's law curve for method C

with 1 mL of 320 µg mL^{-1} NBS in method A, 1.0mL of 340 µg mL^{-1} NBS and 1.0mL of 80 µg mL^{-1} NBS in method C followed by determination of the residual NBS as described under the respective procedures.

For both steps, i.e., oxidation of drug and bleaching of dye by NBS, hydrochloric acid medium was found to be ideal for bleaching of dyes. One mL of 5 M hydrochloric acid for method A and 1 mL of 2M HCl for method B, and 1 mL of 1M HCL in a total volume of ~3-4 mL was adequate for the oxidation step. Which was complete in 5, 15, and 15 min in method A, method B and method C, respectively, and the same quantity of acid was employed for the estimation of the dye. The contact time is not critical and any delay up to 30 min had no effect on the absorbance.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration of GTF.

The graphs showed negligible intercept and are described by the equation:

$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in $\mu g \ mL^{-1}$). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [28] are also presented in Table. 1 and reveal the very high sensitivity of the proposed methods.

Method Validation

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the methods (Table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions

being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 2.3-3.5% and represent the best appraisal of the methods in routine use.

Application to analysis of commercial samples

In order to check the validity of the proposed methods, GTF was determined in some commercial formulations. Table 3 gives the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student's t- test for accuracy and variance ratio F- test for precision with those of the reference method [9] at 95 % confidence level. The calculated t- and F-values (Table 3) did not exceed the tabulated values (t=2.77, F=6.39), except in a couple of instances, for four degrees of freedom indicate that there was no significant difference between the proposed methods and the reference method in respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure GTF at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination. The results of recovery study are compiled in Table 4.

Conclusions

Three useful micro methods for the determination of GTF have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. The proposed methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and

Table 1.Analytical and regression parameters of proposed methods

Parameter	Method A	Method B	Method C
λ_{max}, nm	550	610	520
Beer's law limits, $\mu g \ mL^{-1}$	0.5 – 10	0.5 – 7.5	0.5 – 1.5
Molar absorptivity, $L \ mol^{-1} \ cm^{-1}$	3.4×10^4	2.9×10^4	1.8×10^5
Sandell sensitivity, $\mu g \ cm^{-2}$	0.012	0.014	0.002
Limit of detection, $\mu g \ mL^{-1}$	0.08	0.12	0.02
Limit of quantification, $\mu g \ mL^{-1}$	0.24	0.36	0.06
Regression equation, Y*			
Intercept (a)	0.0050	0.0100	0.0227
Slope (b)	0.0832	0.0674	0.3989
Correlation coefficient, (r)	0.9959	0.9975	0.9946
S_a	0.030	0.0125	0.0563
S_b	0.005	0.0024	0.0426

*Y = a+bX, where Y is the absorbance and X concentration in $\mu g \ mL^{-1}$

S_a= Standard deviation of intercept.

S_b= Standard deviation of slope.

Table 2. Evaluation of accuracy and precision

Method*	GTF taken	GTF Found**	Range	RE %	SD	SEM	RSD %	ROE*** %
	3.0	2.98	0.14	0.75	0.05	0.02	1.66	±1.66
A	5.0	4.94	0.12	1.28	0.05	0.02	0.95	±0.95
	7.0	6.89	0.18	1.58	0.05	0.02	0.78	±0.78
	2.0	2.02	0.10	1.05	0.04	0.01	1.73	±1.73
B	4.0	3.93	0.20	1.78	0.06	0.02	1.64	±1.64
	6.0	6.03	0.27	0.52	0.09	0.04	1.54	±1.54
	0.5	0.49	0.01	1.70	0.01	0.002	0.96	±0.96
C	1.0	0.99	0.02	1.48	0.01	0.003	0.86	±0.86
	1.5	1.48	0.04	1.45	0.02	0.006	1.15	±1.15

RE relative error; SD. Standard deviation; SEM. Standard error of mean; RSD. Relative standard deviation; ROE. Range of error.

* The quantities are in $\mu g \ mL^{-1}$, ** Mean value of seven determinations, *** At the 95% confidence level for 6 degrees of freedom.

Table 3. Results of determination of gatifloxacin in formulations and statistical comparison with the reference method

Tablet brand name[#]	Nominal amount, mg	% Found* ± SD			
		Reference method	Method A	Method B	Method C
GAITY[a]	400	100.6±0.55	98.5±1.05 t=4.15 F=3.64	101.1±1.03 t=1.00 F=3.51	99.5±1.1 t=2.11 F=4.00
GATIQUIN[b]	200	99.8±0.81	98.5±1.01 t=2.26 F=1.55	101±1.02 t=2.07 F=1.59	98.2±0.55 t=3.72 F=2.17
G-CEBRAN[c]	400	101.6±0.65	99.5±1.02 t=3.97 F=2.46	100.2±1.33 t=2.23 F=4.19	101±0.98 t=1.16 F=2.27

*Mean value of five determinations; #Marketed by: a. Reddy's Ltd.; b. Cipla Ltd.; c. Blue Cross Ltd.;
Tabulated t-value at 95% confidence level is 2.77; Tabulated F-value at 95% confidence level is 6.39.

Table 4. Results of recovery experiments by standard addition method

Formulation studied	Method A				Method B				Method C			
	Amount of drug in tablet, μg	Amount of pure drug added, μg	Total found, μg	Pure drug recovered *, %	Amount of drug in tablet, μg	Amount of of pure drug added μg	Total found, μg	Pure drug recovered *, %	Amount of drug in tablet, μg	Amount of pure drug added, μg	Total found, μg	Pure drug recovered *, %
GAITY	19.7	10	30.01	103.1	20.22	10	30.45	102.3	1.99	4	6.09	102.5
	19.7	40	60.30	101.5	20.22	20	40.18	99.80	1.99	8	10.09	101.2
	19.7	70	90.33	100.9	20.22	40	60.34	100.3	1.99	12	14.17	101.5
G-CEBRAN	19.9	10	29.87	99.7	20.04	10	30.36	103.2	2.02	4	5.99	99.2
	19.9	40	59.22	98.3	20.04	20	39.68	98.2	2.02	8	9.90	98.5
	19.9	70	90.32	100.6	20.04	40	60.24	100.5	2.02	12	14.04	100.2

*Mean value of three determinations

expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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