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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
Vol. 4, No. 2, pp 154-161, April 2007

Sensitive Spectrophotometric Methods for the Determination of Gatifloxacin in Pharmaceuticals Using Bromate-Bromide, Methylene Blue and Rhodamine-B as Reagents

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Received 6 October 2006; Revised 5 November 2006; Accepted 10 December 2006

Abstracts: Two new simple, precise, rapid and extraction-free spectrophotometric methods are proposed for the determination of gatifloxacin(GTF) using bromate-bromide mixture and two dyes, methylene blue and rhodamine B, as reagents. Spectrophotometric methods entail the addition of a known excess of bromate-bromide mixture to GTF in hydrochloric acid medium followed by determination of residual bromine by reacting with a fixed amount of either methylene blue and measuring the absorbance at 665 nm (Method A) or rhodamine B and measuring the absorbance at 555 nm (Method B). Beer's law is obeyed in the ranges, 0.5-5.0 and 0.2-1.5 μg mL^{-1} for method A and method B, respectively. The apparent molar absorptivities are calculated to be $5.6x10^4$ and $9.6x10^4$ L $mol^{-1}cm^{-1}$ for method A and method B, respectively, and the corresponding Sandell sensitivity values are 0.0071 and 0.0042 μg cm^{-2}. The methods were successfully applied to the assay of GTF in pharmaceutical formulations with satisfactory results.

Keywords: Gatifloxacin, assay, spectrophotometry, bromate-bromide, tablets.

Introduction

Gatifloxacin(GTF) is a synthetically derived,broad spectrum fluoroquinolone designed for both oral and intravenous administration. Chemically, it is (±)-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.

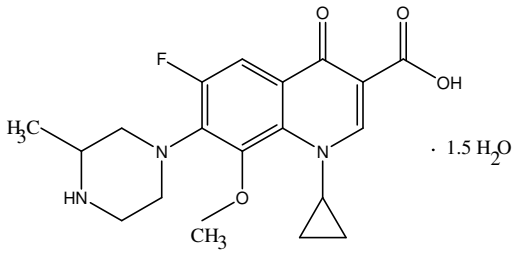

Figure 1. Structure of gatifloxacin sesqui hydrate

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 μg 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] There are two reports[17,18] on the use of N-bromosuccinimide(NBS) as an oxidimetric reagent for the estimation of GTF. The methods are based on the determination of unreacted NBS with celestine blue or by charge transfer reaction involving metol and sulphanilamide. A similar method but using chloramine-T and gallocyanine[18] as reagents is also found in the article. Three sensitive methods [19] based on chloroform extractable ion-association complexes formed by GTF with wool fast blue BL, Tropaeolineooo 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 use of an unstable reagent [17,18], poor sensitivity [14-16] or liquid-liquid extraction step [16,19].

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 bromate-bromide mixture as oxidimetric/brominating reagent, which has successfully been used for the sensitive spectrophotometric determination of many bioactive substances [20-28]. 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

Reagents and materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. A stock standard solution equivalent to 1000 μgmL^{-1} $KBrO_3$ containing a large excess of KBr was prepared by dissolving accurately weighed 100 mg of $KBrO_3$ (Sarabhai M. chemicals, Baroda, India) and 1.0 g of KBr (S.d. Fine Chem. Ltd., Mumbai, India) in water and diluting to 100 mL in a volumetric flask. The above solution was diluted appropriately with water to get 25 and 10 μg mL^{-1} in $KBrO_3$ concentrations for method A and method B, respectively. To prepare 40 μg mL^{-1} methylene blue for method A, first, a 400 μg mL^{-1} dye solution was prepared by dissolving 57 mg of dye (Qualigen fine-chem., Mumbai, assay 70%) in water and diluting to 100 mL in a calibrated flask, and filtered using glass wool. This was appropriately diluted to get the required concentration. For method B, first, a 500 μg mL^{-1} rhodamine B solution was prepared by dissolving 62.5 mg of dye (s.d.fine-chem Ltd., Mumbai, 80 % assay) in water and diluting to 100 mL, and filtered. This was appropriately diluted with water to get 50 μg mL^{-1}. Hydrochloric acid (5M) was prepared by diluting 43 mL of concentrated acid (s.d.fine-chem Ltd., Mumbai, Sp gr 1.18) to 100 mL with water. Pharmaceutical grade GTF, certified to be 99.85 % pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A 500 μg mL^{-1} stock solution of GTF was prepared by dissolving accurately weighed 50 mg of pure drug in water with the aid of heat and diluted to 100 mL with water in a calibrated flask. This was diluted with water to get working concentrations of 10 and 5 μg mL^{-1} GTF for method A and method B, respectively.

Method using methylene blue (method A)

Aliquots of pure GTF solution (0.5 to 5.0 mL; 10 μg mL^{-1}) were accurately measured and transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 5.0 mL with water. To each flask were added 1 mL of 5M hydrochloric acid followed by 1 mL of bromate-bromide mixture (25 μg mL^{-1} in $KBrO_3$).The flasks were closed, the content was mixed well and the flasks were set aside for 10 min with occasional shaking. Finally, 1 mL of 40 μg mL^{-1} methylene blue solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 665 nm against reagent blank after 10 min.

Method using rhodamine B (method B)

Varying aliquots (0.5-3.0 mL) of 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 3.0 mL with water. To each flask were added 1 mL each of 5 M hydrochloric acid and bromate-bromide mixture (10 μg mL^{-1} in $KBrO_3$) successively; the flasks were closed and let stand for 10 min with occasional shaking. Then, 1 mL of 50 μg mL^{-1} rhodamine B 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 555 nm against a reagent blank after 10 min.

In either spectrophotometric method, 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 50 mg of GTF was accurately weighed into a beaker, 50 mL water was added and stirred for 20 min and warmed. Then, the content of the beaker was quantitatively transferred into a 100 mL calibrated flask, diluted to the mark with water and 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 (500 µg mL^{-1} GTF) was diluted appropriately to get 10 and 5 µg mL^{-1} concentrations for analysis by method A and method B, respectively.

Results and Discussion

The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (*insitu* generated) after allowing the reaction between GTF and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either methylene blue or rhodamine -B dye. The methods rely on the bleaching action of bromine 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 *insitu* generated bromine, consumes the latter proportionately and there occurs a concomitant fall in the amount of bromine. When a fixed amount of dye is added to decreasing amounts of bromine, 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 and 3).

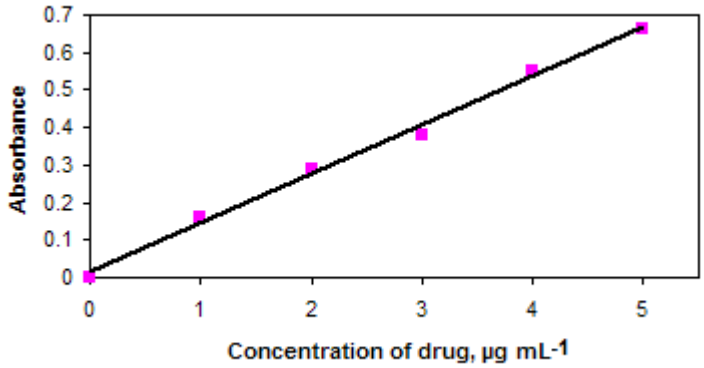

Figure 2. Beer's law curve for method A

Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these were found to be 4 and 5 µg mL^{-1} for methylene blue and rhodamine B, respectively. A bromate concentration of 2.5 µg mL^{-1} was found to irreversibly destroy the blue colour of 4 µg mL^{-1} methylene blue whereas 1.0 µg mL^{-1} bromate in the presence of a large excess of bromide was required to bleach red colour due to 5 µg mL^{-1} rhodamine B. Hence, different amounts of GTF were reacted with 1 mL of 25 µg mL^{-1} bromate in method A and 1mL of 10 µg mL^{-1} bromate in method B followed by determination of the residual bromine as described under the respective procedures.

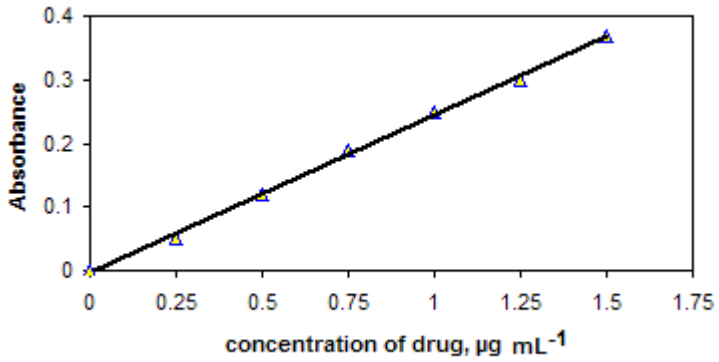

Figure 3. Beer's law curve for method B

For both steps, i.e., bromination of drug and bleaching of dye by bromine, hydrochloric acid medium was found to be ideal. One mL of 5 M hydrochloric acid in a total volume of about~3-5 mL was adequate for the bromination step which was complete in 10 min in both methods and the same quantity of acid was employed for the estimation of the dye. Contact time of 10 min is not critical and any delay upto 30 min, had no effect on the absorbance in both methods.

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 µg 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 [29] are also presented in Table. 1 and reveal the very high sensitivity of the spectrophotometric 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 2.5-3.5% and represent the best appraisal of the methods in routine use.

Table 1. Analytical and regression parameters of the proposed methods

Parameter	Method A	Method B
λ_{max}, nm	665	555
Beer's law limits, (μg mL^{-1})	0. 5 –5.0	0.2 – 1.5
Molar absorptivity, (L mol^{-1} cm^{-1})	5.6×10^4	9.6×10^4
Sandell sensitivity, (μg cm^{-2})	0.0071	0.0042
Limit of detection, (μg mL^{-1})	0.07	0.05
Limit of quantification, (μg mL^{-1})	0.21	0.16
Regression equation, Y*		
Intercept (a)	0.0300	-0.0067
Slope (b)	0.1260	0.2514
Correlation coefficient, (r)	0.9965	0.9986
S_a	0.0193	0.0069
S_b	0.0061	0.0066

*Y = a+bX, where Y is the absorbance and X concentration in μg mL^{-1}
S_a= Standard deviation of intercept.
S_b= Standard deviation of slope.

Table 2. Evaluation of accuracy and precision

Method	GTF taken, (μg mL^{-1})	GTF Found*, (μg mL^{-1})	Range, (μg mL^{-1})	RE (%)	SD, (μg mL^{-1})	SEM, (μg mL^{-1})	RSD, (%)	ROE, ** (%)
Method A	1.5	1.48	0.01	1.59	0.022	0.008	1.46	±1.46
	2.5	2.47	0.04	1.17	0.029	0.011	1.18	±1.18
	3.5	3.46	0.19	1.25	0.063	0.024	1.82	±1.82
Method B	0.5	0.49	0.01	1.21	0.004	0.002	0.84	±0.84
	1.0	0.99	0.02	1.09	0.008	0.003	0.83	±0.83
	1.5	1.48	0.06	1.10	0.019	0.007	1.31	±1.31

RE. relative error; SD. Standard deviation; SEM. Standard error of mean; RSD. Relative standard deviation; ROE. Range of error;
* Mean value of seven determinations
** At the 95% confidence level for 6 degrees of freedom.

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 the Student's t- test for accuracy and the 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.

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
GAITY[a]	400	100.6±0.55	99.8±1.05 t=1.58 F=3.64	101.1±1.03 t=1.00 F=3.51
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
G-CEBRAN[c]	400	101.6±0.65	99.95±1.02 t=3.12 F=2.46	101.9±1.33 t=0.48 F=4.19

*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

Tablets studied	Method A				Method B			
	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 pure drug added (µg)	Total found (µg)	Pure drug recovered* (%)
GAITY	19.96	10	30.08	101.2	5.06	2.5	7.62	102.3
	19.96	20	40.46	102.5	5.06	5.0	10.05	99.8
	19.96	30	50.23	100.9	5.06	7.5	12.58	100.3
G-CEBRAN	20	10	29.97	99.7	5.1	2.5	7.68	103.2
	20	20	39.66	98.3	5.1	5.0	10.01	98.2
	20	30	50.18	100.6	5.1	7.5	12.64	100.5

*Mean value of three determinations

Conclusions

Two useful micro methods for the determination of GTF have been developed and validated as per the ICH guidelines. The methods are simple and rapid taking not more than 15-20 min for the assay. Both spectrophotometric 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 expensive technique like HPLC.

Both methods employ a solution which is quite stable in solution unlike many reported visible spectrophotometric procedures where the oxidimetric reagents require daily standardization. Compared to reported spectrophotometric methods (both UV and visible) the present methods are highly sensitive and in fact, method B (ε=9.6x10^4 L mol^{-1} cm^{-1}) is the most sensitive ever reported for gatifloxacin. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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