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Sensitive Spectrophotometric Methods for Quantitative Determination of Gatifloxacin in Pharmaceutical Formulations using Bromate-Bromide, Thiocyanate and Tiron as Reagents

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Abstract. Two simple, sensitive and rapid methods are described for the determination of gatifloxacin sesqui hydrate (GTF) in bulk drug and in formulations using bromate-bromide as the oxidimetric reagent. The methods are based on the oxidation of GTF by *in situ* generated bromine followed by determination of unreacted bromine by two different reactions. In one procedure, the residual bromine is reduced by an excess of iron(II), and the resulting iron(III) is complexed either with thiocyanate and measured at 470 nm (method A) or with tiron at pH 1.09 and measured at 670 nm (method B). In both methods, the absorbance is found to decrease linearly with GTF concentration. Beer's law is obeyed over the ranges 0.3-3.0 and 1-15 μg/mL for method A and method B, respectively. The calculated molar absorptivity values are 1.3×10^5 and 2.5×10^4 L/mol/cm for method A and method B, respectively. The methods were successfully applied to the determination of GTF in formulations and the results tallied well with the label claim. The results were statistically compared with those of a literature method by applying the Student's t-test and F-test. No interference was observed from the concomitant substances normally added to preparations. The accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard-addition method.
Keywords. Gatifloxacin, Spectrophotometry, Complexation, Pharmaceuticals, bromate-bromide.

Resumen. Se describen dos métodos simples, sensibles y rápidos para la determinación del sesquihidrato de gatifloxacina (GTF) como droga cruda y en formulaciones, empleando bromuro-bromato como el reactivo oxidimétrico. Los métodos se basan en la oxidación de GTF por bromo generado *in situ* seguido de la determinación del bromo excedente por dos reacciones diferentes. En un procedimiento, el bromo residual se reduce por exceso de Fe(II), y el Fe (III) resultante se compleja, ya sea con tiocianato y medido a 470 nm (método A) o con tiron a pH 1.09 y medido a 670 nm (método B). En ambos métodos se encuentra que la absorbancia decrece linealmente con la concentración de GTF. Se sigue la ley de Beer en los rangos de 0.3 – 3.0 y 1 – 15 μg / mL por el método A y por el método B, respectivamente. Los valores calculados de absorptividad molar son 1.3×10^5 y 2.5×10^4 L /mol /cm para los métodos A y B, respectivamente. Los métodos fueron aplicados exitosamente a las determinaciones de GTF en formulaciones y los resultados coincidieron con lo informado en la etiqueta. Los resultados fueron comparados estadísticamente con los informados en la literatura empleando pruebas t de student y la prueba F. No se observaron interferencias con las substancias empleadas para el análisis. La exactitud y validez de los métodos fueron confirmados por experimentos de recuperación mediante el método estándar de adición.
Palabras clave: Gatifloxacina, espectrofotometría, complejación, farmacéuticos, bromato-bromuro.

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]. 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 [7] for the assay of GTF in bulk drug and dosage forms. A non-aqueous titration procedure [8] has recently been described for the assay of drug in pharmaceutical formulations using perchloric acid as titrant. Very recently, Salgado *et al*. [9] have reported a microbiological assay for GTF in pharmaceutical formulations. Several UV-spectrophotometric [10-14] 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*. [15] 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 [16] for the determination of drug in 40-160 μg mL^{-1} range in dosage forms. Two methods, one based on redox-complexation reaction involving chromium (VI) and sym-diphenylcarbazide and the other on Mannich reaction, have recently been reported by Saraswathi *et al*. [17]. There are two reports [18,19] 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 chloramines-T and gallocyanine [19]

as reagents is also found in the article. Three sensitive methods [20] 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 use of an unstable reagent [18,19], poor sensitivity [15-17] or liquid-liquid extraction step [17,20].

The objective of this investigation was to devise simple, rapid, sensitive and economically viable procedures that could be used to determine GTF in bulk drug and pharmaceutical dosage forms. The methods rely on the use of bromate-bromide as the oxidimetric reagent, and iron (II) and thiocyanate or tiron as the subsidiary reagents. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, cost-effectiveness and eco-friendliness.

Experimental

Apparatus

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

Materials

All chemicals were of analytical reagent grade and distilled water used to prepare solutions.

Bromate-bromide mixture (20 and 150 µg/mL in $KBrO_3$). A stock standard solution equivalent to 1000 µg/mL $KBrO_3$ and a large excess of KBr was first prepared by dissolving accurately weighed 100 mg of $KBrO_3$ and 1g of KBr in water and diluting to the mark with water in a 100 mL calibrated flask. This was diluted stepwise to obtain working concentrations containing 20 and 150 µg/mL $KBrO_3$ for use in method A, and method B, respectively.

Hydrochloric acid**.** 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 diluted further to get 1 M for use in method B.

Ferrous ammonium sulphate, FAS (400 and 1400 µg/mL). A stock solution equivalent to 0.01 M FAS was prepared by dissolving about 400 mg of the salt (S.d. Fine Chem, Mumbai, India) in 50 mL of water containing 1mL of dil H_2SO_4 , and diluted to 100 mL with water, and standardized [21] using pure potassium dichromate. The stock solution was then diluted appropriately with water to get 400 and 1400 µg/mL FAS for method A and method B, respectively.

Tiron (1.0%). About 1.0 g of Tiron (Loba Chemie, Mumbai, India) was dissolved in 100 mL of water.

Ammonium thiocyanate (3 M). Prepared by dissolving 23 g of the chemical (S.d. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

Sodium acetate tri hydrate(1.5 M): Prepared by dissolving 24.5 g of the chemical (S.d. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

Buffer of pH 1.09: Prepared by mixing of 50 mL 1 M sodium acetate and 70 mL of 1 M HCl and diluting to 250 mL with water.

Standard drug solution. Pharmaceutical grade GTF, certified to be 99.85% pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A 1 mg 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 mg mL^{-1}) was diluted with water to get working concentrations of 10 and 50 mg mL^{-1} GTF for method A and method B, respectively.

Procedures

Method A

Different aliquots (0.3-3.0 mL) of standard 10 µg/mL GTF solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 3.0 mL by adding water. To each flask was added 1mL each of 5 M HCl and bromate-bromide (20 µg/mL w.r.to $KBrO_3$), the last being added using micro burette. The flasks were stoppered, the content was mixed and the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 400 µg/mL FAS was added to each flask (micro burette), and again the flasks were let stand for 5 min followed by 1 mL of 3 M thiocyanate. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 470 nm against water blank.

Method B

Varying aliquots (0.2-3.0 mL) of standard GTF solution (50 µg/mL) were accurately measured into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. The solution in each flask was acidified by adding 1 mL of 1 M HCl before adding 1mL of bromate-bromide (150 µg/mL w.r.to $KBrO_3$) by means of micro burette. The flasks were stoppered, the content was mixed well and allowed to stand for 15 min with occasional shaking. To each flask was then added 1mL of 1400 µg/mL FAS, and after 5 min, 1 mL each of 1.5 M sodium acetate, buffer of pH 1.09 and 1% tiron were added and diluted to the mark with water. The absorbance of each solution was measured at 670 nm against water blank.

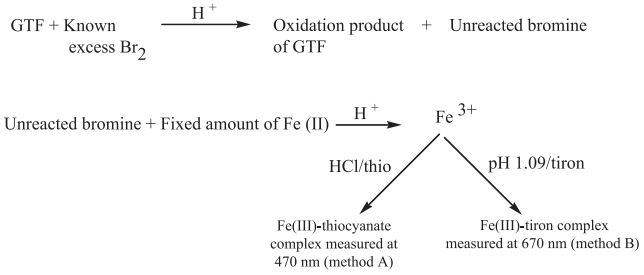
In either spectrophotometric method, a standard graph was prepared by plotting the decreasing absorbance values versus concentration of GTF. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer's law data.

Procedure for Tablets

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 content was quantitatively transferred into a 100 mL calibrated flask, the beaker was washed with water and washings were transferred to the flask and the volume was made up to the mark 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 10 and 50 mg mL^{-1} concentrations for analysis by method A and method B, respectively.

Results and Discussion

The proposed methods are indirect and are based on the determination of residual bromine after allowing the reaction between GTF and oxidant to go to completion, and rely on two different well-known reactions.



Method Development

The methods are based on the oxidation of GTF by a known excess of *in situ* generated bromine in hydrochloric acid medium, reducing the unreacted oxidant by iron (II) and subsequent determination of iron (III) by thiocyanate method [22] or by tiron method of Potter and Armstrong [23] and modified by Keshavayya *et al*. [24] When a fixed concentration of bromate-bromide is made to react with increasing concentration of GTF in HCl medium, there occurs a concomitant fall in the concentration of bromine. When the unreacted oxidant is reduced by a fixed concentration of iron(II), there will be a proportional decrease in the concentration of iron (III). This is observed as a proportional decrease in the absorbance of iron (III) – thiocyanate complex and iron(III)-tiron complex on increasing the concentration of GTF (Fig. 2 and 3), which formed the basis for the determination of drug.

Various parameters associated with the oxidation of GTF by *in situ* bromine and subsequent reduction of the residual oxidant by iron (II) were optimized. Considering 5.5 μg/mL as the upper limit of iron that could be determined by thiocyanate method, 20 μg/mL bromate in the presence of excess of bromide was found to produce it from 400 μg/mL FAS in method A (Fig. 4). Similarly in method B, fixing 18 μg/mL as the upper limit of iron that could be determined by tiron method, 1400 μg/mL FAS and 150 μg/mL bromate with excess bromide were used (Fig.5). One mL of 5 M HCl in a total volume of 6 mL was used for the oxidation step and the same quantity of acid was used for the reduction of oxidant and complexation of iron (III) with thiocyanate in method A. However, the formation of iron(III)-tiron complex(1:1) is pH dependent and 1 mL of 1 M HCl in a total volume of ~5 was used to cause

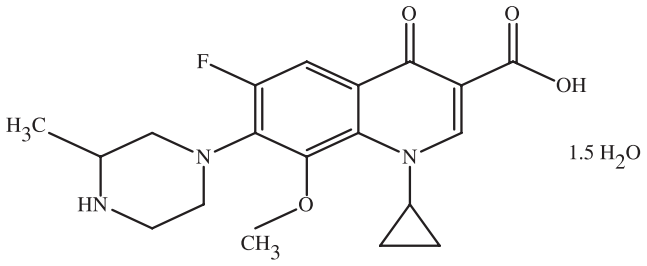


Fig. 1. Structure of gatifloxacin sesqui hydrate

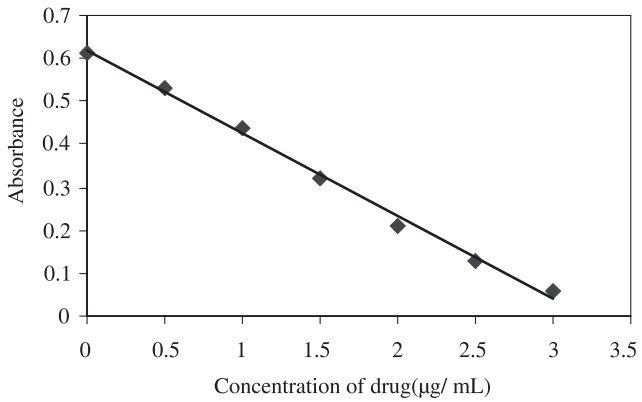


Fig. 2. Beer's law curve for method A.

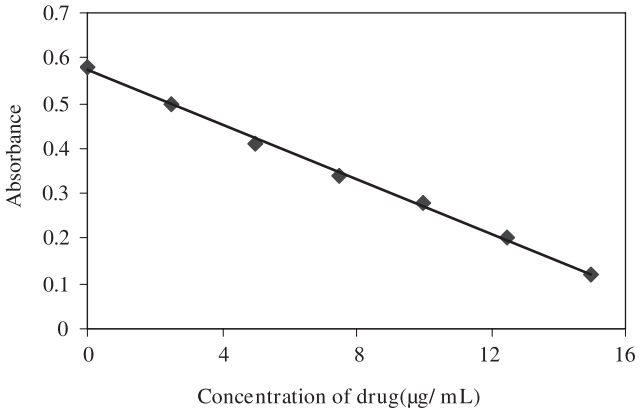


Fig. 3. Beer's law curve for method B.

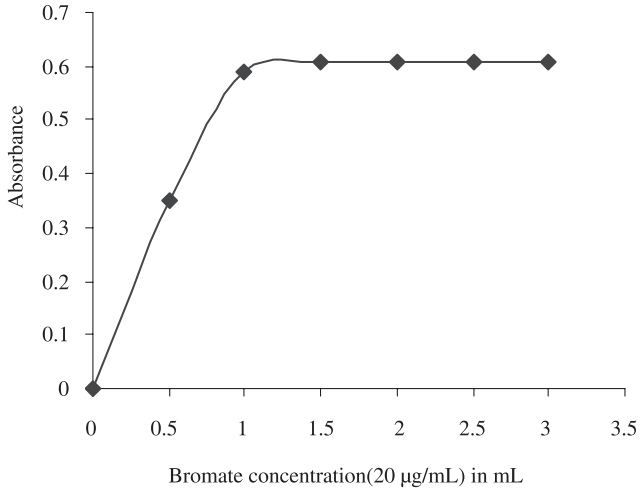


Fig. 4. Optimization of bromate-bromide for method A.

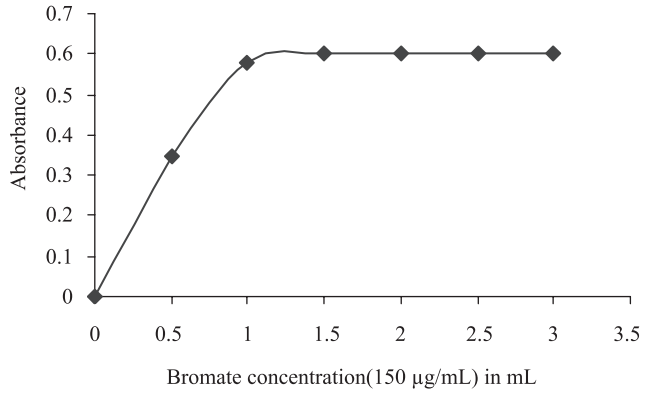


Fig. 5. Optimization of bromate-bromide for method B.

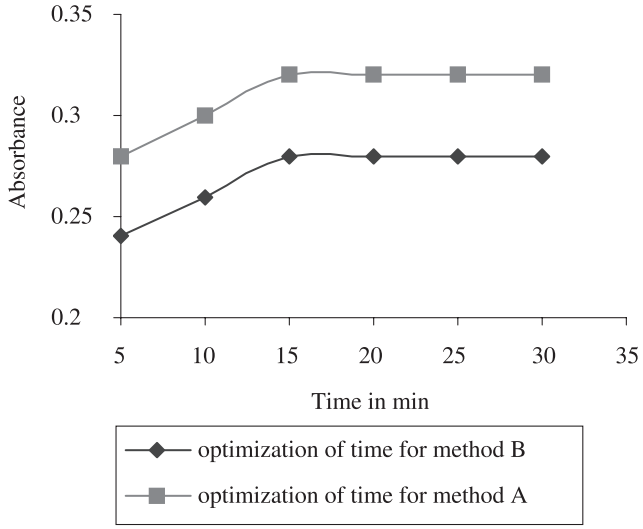


Fig. 6. Optimization of reaction time for method A and B.

oxidation of drug by *in situ* bromine and the latter's reduction by iron(II), and later the pH was raised to ~1.0 by adding 1.0 mL of 1.5 M sodium acetate solution. To ensure an optimum pH for the complex formation reaction, 1 mL of buffer of pH 1.09 was also added. The oxidation of GTF was complete in 5-10 min but the reduction of oxidant by iron(II) and subsequent complexation of iron(III) with thiocyanate or tiron were instantaneous.

Analytical parameters

A linear relation is found between absorbance and concentration in the ranges given in Table 1. In both methods, Beer's law is obeyed in the inverse manner. The calibration graphs are described by the equation:

$$Y = a + b\,X$$

Table 1. Analytical and regression parameters of the proposed methods.

Parameter	Method A	Method B
λ_{max}, nm	470	670
Beer's law limits, mg /mL	0.3 –3.0	1-15
Molar absorptivity, L/ mol/ cm	1.3×10^5	2.5×10^4
Sandell sensitivity, µg /cm^2	0.0032	0.0158
Limit of detection, mg /mL	0.05	0.22
Limit of quantification, mg /mL	0.15	0.66
Regression equation, Y* Intercept (a) Slope (b)	0.6207 -0.1937	0.5673 -0.030
Correlation coefficient, (r)	-0.9960	-0.9987
S_a	0.0181	0.0080
S_b	0.0087	0.0008

*Y = a+bX, where Y is the absorbance and X concentration in mg /mL
S_a= Standard deviation of intercept.
S_b= Standard deviation of slope.

(where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in Table 1. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification calculated according to ICH guidelines [25] are also compiled in Table 1, and demonstrate the high sensitivity of the methods.

Method Validation

Evaluation of accuracy and precision. Intra-day and inter-day precision were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for seven replicate analyses at three different concentration levels were calculated. The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE). To determine the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. Table 2 summarizes the intra-day precision and accuracy data for the determination GTF by the proposed methods.

Application

Table 3 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a literature method [10] by applying Student's t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39), suggesting that the proposed methods are as accurate and precise as the reference method.

Accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard addition technique. To a fixed and known amount of GTF in tablet (pre-analysed), pure drug was added at three levels and the total was found by the proposed methods (Table 4). Each test was repeated three times. The recovery of pure GTF added to tablet indicates that commonly encountered excipients and additives such as talk, starch, lactose, sodium alginate, magne-

Table 2. Evaluation of accuracy and precision.

Method	GTF taken, µg /mL	GTF Found*, µg /mL	Range, µg /mL	RE %	SD, µg /mL	SEM, µg /mL	RSD, %
Method A	0.5 1.5 2.5	0.49 1.49 2.48	0.01 0.08 0.10	2.00 0.67 0.80	0.009 0.018 0.021	0.003 0.007 0.008	1.84 1.21 0.85
Method B	4.0 8.0 12.0	3.96 7.98 11.92	0.07 0.05 0.06	1.00 0.25 0.67	0.066 0.085 0.112	0.025 0.032 0.042	1.67 1.07 0.94

RE. relative error; SD. Standard deviation; SEM. Standard error of mean; RSD. Relative standard deviation.
* Mean value of seven determinations

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

Tablet brand name#	Nominal amount, mg	% Found* ± SD		
		Literature method	Method A	Method B
GAITY[a]	400	100.3±0.52	99.5±0.69 t = 2.09 F = 1.76	100.8±0.56 t = 1.46 F = 1.16
GATIQUIN[b]	200	99.9±0.62	99.2±1.01 t = 1.36 F = 2.65	100.2±1.02 t = 0.58 F = 2.71
G-CEBRAN[c]	400	99.8±0.62	100.5±1.32 t = 1.14 F = 4.53	99.2±1.33 t = 0.97 F = 4.60

*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.

sium stearate, calcium gluconate and calcium dihydrogen orthophosphate did not interfere in the assay procedures.

Conclusions

Two new visible spectrophotometric methods for the assay of gatifloxacin in pharmaceuticals using bromate-bromide as the oxidimetric reagent have been developed and appropriately validated. Both methods are based on well characterised complexation reactions and are the most sensitive ever reported for GTF in pharmaceuticals (Table 5). The stability of the coloured species and sensitivity of the reactions used are not critically dependent on any experimental variable unlike in many reported methods. Results of assay of authentic samples indicate non-interference from tablet excipients. Besides, the methods are reasonably accurate and precise and hence can be conveniently used in routine use.

Table 4. Results of recovery experiments by standard addition method

Tablets studied	Method A				Method B			
	Amount of drug in tablet, µg /mL	Amount of pure drug added, µg /mL	Total found µg /mL	Pure drug recovered*, %	Amount of drug in tablet, µg /mL	Amount of pure drug added, µg /mL	Total found, µg /mL	Pure drug recovered* %
GAITY	0.50	0.50	0.99	98.2	5.04	2.00	7.03	99.6
	0.50	1.50	2.02	101.2	5.04	4.00	9.06	100.5
	0.50	2.00	2.51	100.5	5.04	6.00	10.97	98.8
G-CEBRAN	0.50	0.50	1.00	99.4	4.96	2.00	6.95	99.6
	0.50	1.50	1.97	98.2	4.96	4.00	9.03	101.8
	0.50	2.00	2.55	102.5	4.96	6.00	11.09	102.2

*Mean value of three determinations

Table 5. Comparison of the proposed and reported methods

Sl.No.	Reported methods	Reagents used	l_{max}, nm	Range, µg mL^{-1}	LOQ, µg mL^{-1}	Remarks	Ref.
1	HPLC	-	279-295	4-24	-	Applicable combined fluoroquinolonic antibiotics	7
2	Titrimetry	Perchloric acid	-	-	-	Hazardous chemical involved	8
3	Microbiological assay	-	-	-		-	9
4	UV-spectrophotometry	HCl	286	1-18	0.31	pH 7.4 to be maintained	10
			292	1-14	0.3	pH 1.2 to be maintained	
5	UV-spectrophotometry	-	286	2-14	-	Absorbance measured at shorter wavelength	11
6	UV-spectrophotometry	-	-	2.4-6.4	-	-	12
7	UV-spectrophotometry	-	287	4-14	-	Absorbance measured at shorter wavelength	13
8	UV-spectrophotometry	-	284	3-15	-	-	14
9	Visible spectrophotometry	Fe(III)	-	-	-	-	15
10	Visible spectrophotometry	Ceric ammonium sulphate	455	40-160	-	Wide linear range; less sensitive	16
11	Visible spectophotometry	Cr^{6+}-diphenyl carbazide	548	1-10	-	Expensive chemical	17
		Mannich reaction	453	10-40	-	Wide linear range	
12	Visible spectophotometry	NBS	-	2-10	-	NBS requires daily standardisation	18,19
			-	4-20	-		
			-	1-5	-		
13	Visible spectrophotometry	chloroform	590	1-6	-	Extraction involved	20
			480	1-6	-		
			420	2-10	-		
14	Vis- spectrophotometry	**a**)bromate bromide/thiocyanate	470	3-3.0 ($\sum = 1.3 \times 10^5$)	0.15	Highly sensitive, wide linear dynamic ranges, inexpensive instrumental setup, use of eco-friendly chemicals.	Present methods
		b. bromate bromide/tiron	670	0.1-15 $\sum = 2.5 \times 10^4$)	0.66		

$\sum$. Molar absorptivity in L mol^{-1} cm^{-1}
NBS. *N*-bromosuccinimide

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