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Fluorescence probe enhanced spectrofluorimetric method for the determination of gatifloxacin in pharmaceutical formulations and biological fluids

Xiashi Zhu *, Aiqin Gong, Suhai Yu

College of Chemistry & Chemical Engineering, Yangzhou University, Yangzhou 225002, China

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Abstract

A spectrofluorimetry for the determination of gatifloxacin (GFLX) was developed based on the strong fluorescence of gatifloxacin after adding fluorescence probe yttrium in buffer solution (pH 7.0) and various factors of influencing fluorescence have been researched. Under the optimum conditions, the liner range was 4.00×10^{-8} to 1.00×10^{-6} g mL^{-1} and the detection limit is 3.36×10^{-9} g mL^{-1} (correlation coefficient $r = 0.9997$), respectively. The relative standard deviation was 1.1% for 11 measurements of 5.6×10^{-7} g mL^{-1} gatifloxacin standard solution. The mechanism of sensitizing effect of probe was discussed. The proposed method has been successfully applied to determine real samples and the obtained results are in good agreement with the results of HPLC.

Keywords: Gatifloxacin; Spectrofluorimetry; Probe

1. Introduction

Gatifloxacin {±-1-cyclopropyl-6-fluoro-7-(3-methyl-1-piperaziny)-8-methoxy-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid sesquihydrate} is a new broad-spectrum 8-methoxy fluoroquinolone antibacterial agent. Its molecular formula is $C_{19}H_{22}FN_3O_4 \cdot 1.5H_2O$ and its molecular weight is 402.23. The structure of GFLX is shown in Fig. 1.

The new fluoroquinolone, which has shown activity against both Gram (+), Gram (−) and anaerobic species, behaves excellent pharmacokinetic and pharmacodynamic characters. Its negative action is very small and even no photoactive reaction [1–3]. Thus, a rapid, low cost, and accessible procedure for the quantification of gatifloxacin in dosage forms is desirable. Various techniques have been utilized for the determination of the fluoroquino-lone, among them HPLC is the most frequently used technique [4,5]. Other analytical methods are spectrophotometry (UV) [6], capillary electrophoresis [7] and fluorospectrophotometry [1,3,8], FIA-chemiluminescence [9], etc. These methods were limited to widely use because of expensive instruments cost or low sensitivity.

Probes are some small organics, inorganic ions, metal complex ions and quantum points. The spectrophotometric or electrochemical characters of the analytes would change when they form extra-molecular complexes with probes, therefore it could provide concentrative or structural information of the analytes or enhance sensitivity of determination [10]. Nowadays there are many cases to use probes in assaying pharmaceuticals. Literature [11] studied the reaction of oxyfloxacin with DNA using Tb as probe. There was also a paper [12] that used methylene blue as probe to research the reaction of *p*-(*N*,*N*′-dimethyl aminomethyl) calix [8] arene with DNA. Some papers have reported to determine gatifloxacin by spectrofluorimetry without probes [1,8], but the sensitivity was low. The sensitivity of assay gatifloxacin could be enhanced in the medium of SDS micelle solution [3]. Fluorescence probe enhanced spectrofluorimetric method for the determination of GFLX seems to be lacking. In this paper, Y^{3+} as probe, spectrofluorimetric method for the determination of GFLX is investigated. To the best of our knowledge, this is the first attempt to determine GFLX in pharmaceutical formulations by fluorescence probe enhanced spectrofluorimetry. The main advantages of the proposed

* Corresponding author. Tel.: +86 514 7975244; fax: +86 514 7975244.
E-mail addresses: xszhu@yzu.edu.cn, zhuxiashi@sina.com (X. Zhu).

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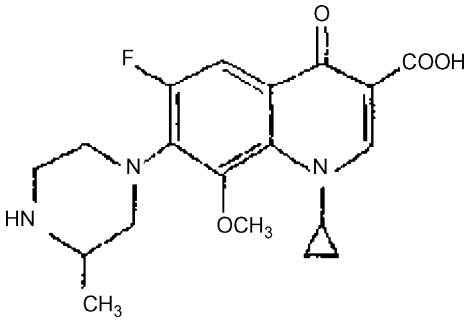

Fig. 1. The structure of gatifloxacin.

methods compared with previously reported are their simplicity and lower cost. The detection limits are comparable or better than the detection limits of the other methods [1,3,8].

2. Experimental

2.1. Chemicals

Gatifloxacin was kindly provided by Jinghua institution. Gatifloxacin capsule and injection were from Biocause (Hunai, China) and Suzhong (Jiangsu, China) pharmaceutical industries, respectively. Acetic acid, ammonium acetate, hydrochloric acid and Y_2O_3 were purchased from reagent company of Guoyao (Shanghai, China). All chemicals were of analytical reagent grade. Water was distilled, deionised.

Gatifloxacin stock standard solution of 2.0×10^{-4} g mL^{-1} was prepared by dissolving 0.0100 g of gatifloxacin in 50 mL of 0.1 mol L^{-1} HCl and kept in the dark. Working standard of 2.0×10^{-5} g mL^{-1} was prepared daily by dilution of stock standard solution with high-purity water. Buffer solution (pH 7.0) was prepared by dissolving 77.0 g ammonium acetate in 500 mL high purity water and was adjusted by the addition of acetate acid. The solution of Y^{3+} was prepared by dissolving 0.1270 g Y_2O_3 in 1 mL of HCl and diluted to 100 mL with water.

2.2. Apparatus

An Hitachi F-4500 spectrofluorimeter (Japan) was used for all the measurement, with excitation and emission slits at 2.5 nm, $\lambda_{exc} = 292$ nm and 1 cm quartz cell. The pH was measured on a pH S-25 pH-meter (Shanghai).

2.3. Sample preparation

The solution of gatifloxacin capsule and gatifloxacin injection were prepared by dissolving suitable amount of the commercial samples in 0.1 mol L^{-1} HCl and diluted the resulting solution to adjust the concentration with water to that required by the experimental conditions adopted.

A 1.0 mL serum sample was deproteinized by adding 4.0 mL 10% trichloroacetic acid (CCl_3COOH) in a centrifuge tube, which was then centrifuged for 15 min at 4000 rpm. The supernatant was diluted with deionized water to suitable concentration. No further pre-treatment was required for urine samples. The treated serum and urine samples were spiked with convenient amounts of GFLX stock solutions. Human serum and urine were kindly provided by healthy volunteers.

2.4. Determination of gatifloxacin

Aliquots of working solutions or sample solutions of gatifloxacin, 3.0 mL of acetic acid–ammonium acetate buffer (pH 7.0), 1.0 mL of Y_2O_3 were pipetted in to 25 mL calibrated flasks and diluted to the mark with water. The obtained solutions were thermostated at 20.0 ± 0.5 °C and the fluorescence intensity was measured, using an excitation wavelength of 292 nm, against a blank solution.

2.5. Determination of relative fluorescence quantum yields

Fluorescence quantum yields of GFLX and GFLX-Y^{3+} were measured using 1.0×10^{-6} g mL^{-1} quinine sulfate as reference material. Under the same apparatus conditions, according to the equation $\varphi_2 = (\varphi_1 A_1 F_2)/(F_1 A_2)$ [13], the quantum yields of the analyte was calculated. Briefly, φ_1 and φ_2 are corresponding the standard and unknown fluorescence quantum yield and F_1 and F_2 the integral areas of two calibration fluorescence emission curves, A_1 and A_2 the absorbance ($\lambda_{absorbance} = \lambda_{emission}$) of the standard and unknown and $\varphi_1 = 0.55$ (25 °C) is known.

3. Results and discussion

3.1. The fluorescence spectra of gatifloxacin

The fluorescence spectra of gatifloxacin in pH 7.0 buffer solution are shown in Fig. 2. Comparing the curves, it was found the fluorescence intensity was enhanced about one time when using 4.0×10^{-5} g mL^{-1} Y^{3+} as probe.

3.2. The selection of probes

The selection of probes is very important in spectrofluorimetry. In this work, probes such as aluminum, terbium, europium,

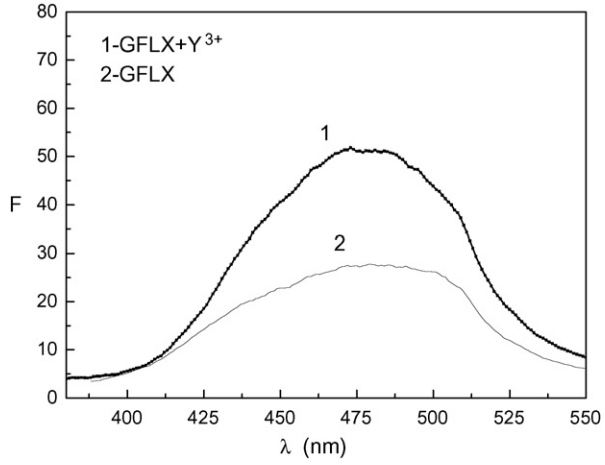

Fig. 2. Fluorescence spectra of GFLX (GFLX: 4.0×10^{-7} g mL^{-1}).

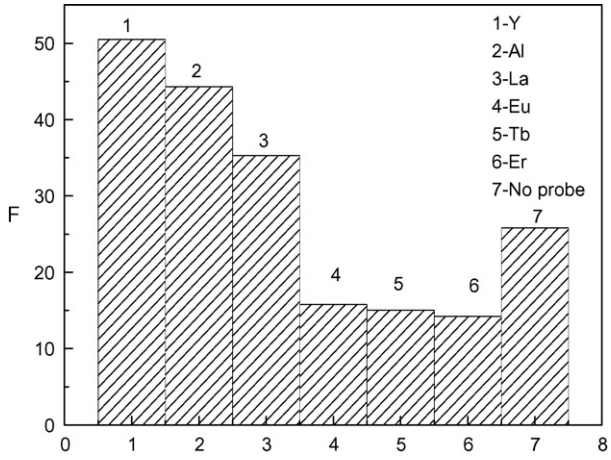

Fig. 3. Effect of different probe on F (GFLX: 4.0×10^{-7} g mL^{-1}).

lanthanum, erbium, yttrium were studied (Fig. 3). The influence of probes on fluorescence intensity (F) could be divided two kinds. One was to enhance gatifloxacin fluorescence intensity such as aluminum, lanthanum or yttrium. The other was to quench fluorescence such as terbium, europium or erbium. Maximum fluorescence intensity was observed in the present of yttrium. The F was approximately one time higher in the presence of probe Y^{3+} than in the absence of Y^{3+}. Hence, yttrium was the probe of choice.

3.3. The effect of pH

The influence of pH on the fluorescence intensity of gatifloxacin was investigated. As could be seen in Fig. 4, the ration of fluorescence intensity with or without Y^{3+} (F_1/F_2) reaches a maximum value at pH 7.0. Moreover, yttrium would be hydrolysised after pH > 8.0. Thus, an acetic acid–ammonium acetate buffer of pH 7.0 was chosen for the determination.

The effect of the value of buffer solution was also studied. Fig. 5 shows that the F_1/F_2 remains stable for a buffer value of 2.0–4.0 mL. So a 3.0 mL buffer solution was selected as suitable for the optimized method (Fig. 5).

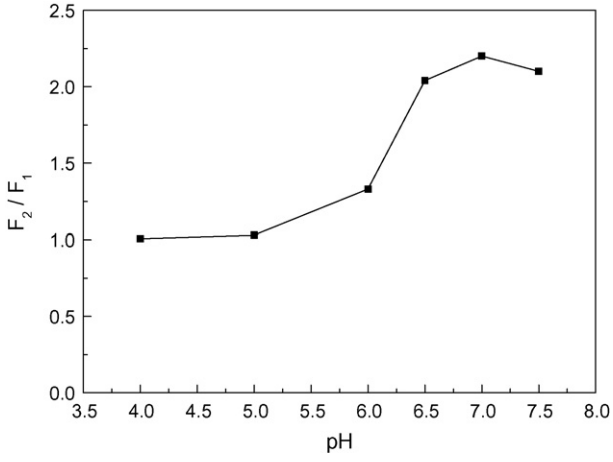

Fig. 4. Effect of pH on the ration of fluorescence intensity (GFLX: 4.0×10^{-7} g mL^{-1}).

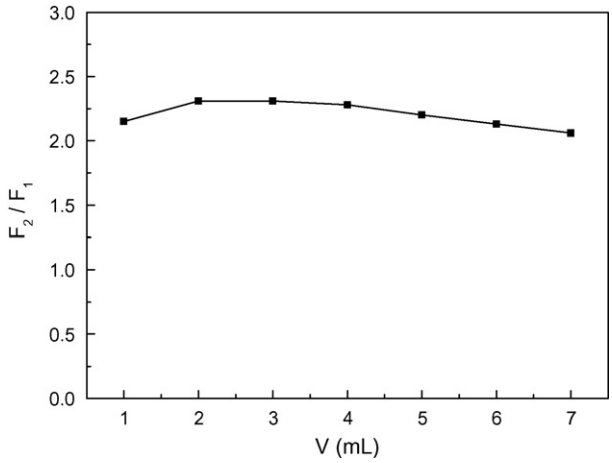

Fig. 5. Effect of $V_{\text{buffer solution}}$ on ration of fluorescence intensity (GFLX: 4.0×10^{-7} g mL^{-1}).

3.4. Effect of probe Y^{3+} concentration

The influence of Y^{3+} concentration on the fluorescence intensity was studied by changing the concentration of Y^{3+}. It was found that 4.0×10^{-5} g mL^{-1} Y^{3+} was appropriate for maximum fluorescence intensity (Fig. 6).

3.5. Effect of temperature

Temperature is an important factor in spectrofluorimetry. As expected, when the temperature is increased the fluorescence intensity is lowered slowly. In this work the fluorescence intensity varied very small about 20 °C, and this temperature was easily kept constant, the temperature of 20 °C (Fig. 7) was selected.

3.6. Effect of ionic strength

The fluorescence intensity remained constant with increasing KCl concentrations up to 0.48 mol L^{-1}; after that, the fluores-

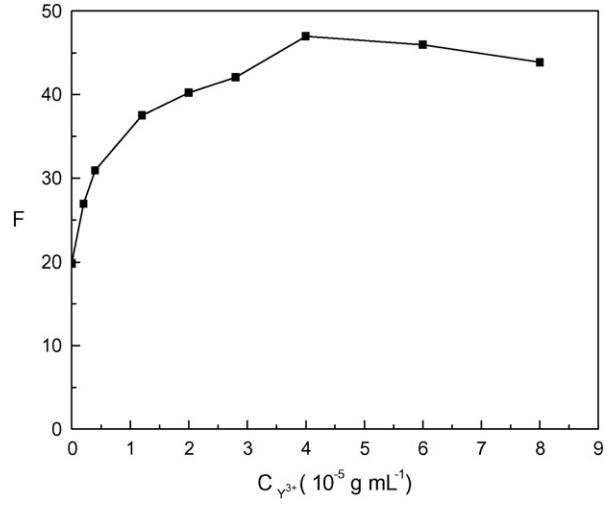

Fig. 6. Effect of Y^{3+} concentration on F (GFLX: 4.0×10^{-7} g mL^{-1}).

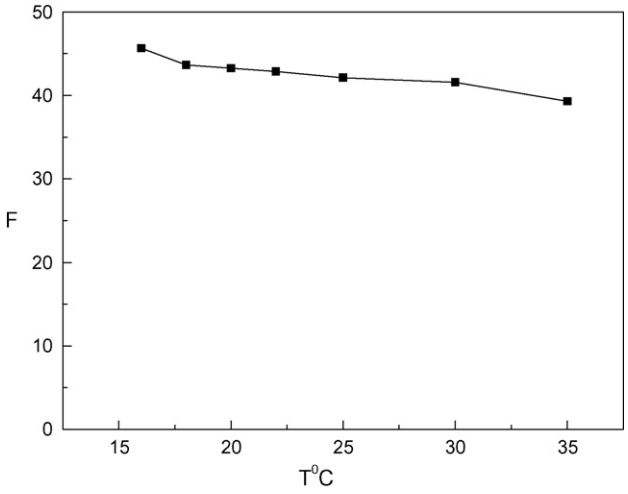

Fig. 7. Effect of temperature on F (GFLX: 4.0×10^{-7} g mL^{-1}).

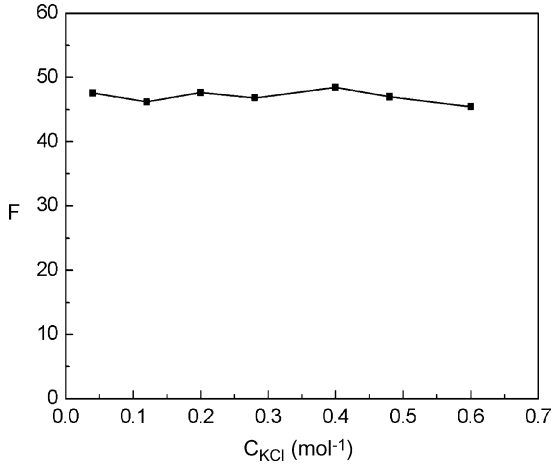

Fig. 8. Effect of ionic strength on F (GFLX: 4.0×10^{-7} g mL^{-1}).

cence intensity decreased. That is, lower ionic strength has no effect on F, while higher ionic strength F decreased, this is because the effect of the electrostatic shielding of charges would reduce the binding of GFLX and Y^{3+} and result in a decreased signal (Fig. 8).

3.7. Discussion of mechanism of sensitizing effect

The fluorescence quantum yields of (Φ_f) in various media were determined, respectively, in order to discuss the influence of the microenvironment on the fluorescence intensity of GFLX. The results are listed in Table 1. The fluorescence quantum yield was one of the mostly basic and significant parameters in all the characters of fluorescence substance [14]. It represented the ability of translating absorption energy to fluorescence. In this paper, the fluorescence quantum yields of GFLX and GFLX-Y^{3+} were determined, respectively. The results were listed in Table 1. The quantum yield Φ_f was approximately 33% times higher in the presence of probe Y^{3+} than in the absence of Y^{3+}. The Φ_f value was tightly related to chemical structure and microenvironment of the system. The Φ_f value is higher, the ability of translating absorption energy to fluorescence is stronger, it is also the reason of enhanced determination sensitivity by Y^{3+}.

Table 1
The results of relative fluorescence quantum yield of GFLX

Sample	Quantum yield Φ_f
GFLX	0.12
GFLX + Y^{3+}	0.16

3.8. Effect of potential interferences

The effects of different foreign substrates were discussed in the determination of 4.0×10^{-7} g mL^{-1} gatifloxacin. The level of tolerated concentrations of foreign ions was considered as maximum concentration found to cause a change in signal, less than ±5%, compared with the signal for gatifloxacin alone. The tolerance limits are shown in Table 2.

3.9. Analytical performance

The calibration graph for the determination of gatifloxacin was obtained under the experimental conditions above described. The results showed that a good linear relationship was observed over the range 4.00×10^{-8} to 1.00×10^{-6} g mL^{-1} .The calibration curve was $F = -1.268 + 129.765C$ (10^{-6} g mL^{-1}), $r = 0.9997$. The detection limit estimated (S/N = 3) was 3.36×10^{-9} g mL^{-1}.

3.10. Analysis of pharmaceutical formulations

The proposed method was applied for the determination of GFLX in real samples. The standard addition method was used to avoid matrix effects and the recoveries are presented in Table 3. The average recoveries obtained by the proposed method were between 97.1 and 103.8% (Table 3).

Table 2
Tolerance limits of interfering ions (mg mL^{-1})

Tested ions	Tolerance limit
Ca^{2+}	0.08
Mg^{2+}	0.08
Ba^{2+}	0.24
Zn^{2+}	0.24
Cd^{2+}	0.12
Hg^{2+}	0.16
Al^{3+}	0.2
Eu^{3+}	0.004
Er^{3+}	0.0032
Tb^{3+}	0.0032
La^{3+}	0.2
Lactose	>8
Cane sugar	>8
Glucose	>8
Urea	3.2
Amylin	3.2
Amylum	3.2

Table 3
Recoveries of GFLX from pharmaceutical products

Sample	Content ($g\,mL^{-1}$)	Added ($g\,mL^{-1}$)	Found ($\times 10^{-6}\,g\,mL^{-1}$)[a]	Recovery (%)	R.S.D. (%)
GFLX capsule	4.0×10^{-7}	8.0×10^{-8}	4.83×10^{-7}	103.8	2.5
		2.4×10^{-7}	6.33×10^{-7}	97.1	1.3
		4.0×10^{-7}	7.99×10^{-7}	99.8	1.1

[a] Average value of three determination.

Table 4
Determination of GFLX in pharmaceutical products

Samples	Batch number	Labeled values	Proposed method[a]	R.S.D. (%, $n=3$)	HPLC method[a]	Relative error[b] (%)
Capsule	050102	0.10 g/particle	0.099 g/particle	0.5	0.0985 g/particle	0.51
Injection	051104031	0.20 g/100 mL	0.202 g/100 mL	2.5	0.199 g/100 mL	1.5

[a] After suitable dilution.
[b] Between results determined by proposed method and HPLC.

Table 5
Results of determination of recovery from biological fluids ($n=3$)

Sample	Added ($g\,mL^{-1}$)	Found ($g\,mL^{-1}$)	Recovery (%)	R.S.D. (%)
Urine	8.0×10^{-8}	8.10×10^{-8}	101.2	1.8
	2.4×10^{-7}	2.32×10^{-7}	96.7	1.1
	4.0×10^{-7}	3.77×10^{-7}	94.3	1.2
Serum	8.0×10^{-8}	7.70×10^{-8}	96.2	2.1
	2.4×10^{-7}	2.34×10^{-7}	97.5	1.7
	4.0×10^{-7}	3.88×10^{-7}	97.0	1.3

In all cases, the results obtained by the proposed method were in good agreement with the labeled amounts (Table 4). In order to further testing the accuracy of the proposed method, the proposed method was compared with HPLC method [15]. The statistical *t*-test was used to compare the results from both methods, which showed that there were not significantly different between them.

3.11. Analysis of spiked serum and urine samples

In order to study the influence of biological fluids on the determination of GFLX, the proposed method was used to determine GFLX in spiked human urine and serum samples. The results are given in Table 5. The recovery was 94.3–101.2% for urine and 96.2–97.5% for serum.

4. Conclusions

A fluorescence probe enhanced fluorospectrophotometry for the determination of GFLX has been developed and successfully applied to the determination of GFLX in pharmaceutical formulations and biological fluids. It shows that proposed yttrium-enhanced fluorimetric method is a simple, fast, accurate and precise procedure.

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