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Determination of Prulifloxacin Using the Eu(III) as the Fluorescence Probe by Spectrofluorimetry

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The fluorescence of the prulifloxacin (PUFX)-Eu(III) system was investigated. Experiments indicated that the fluorescence intensity of Eu(III) could be greatly decreased by prulifloxacin. Accordingly, a sensitive spectrofluorimetric method for the determination of prulifloxacin was established,and its optimum detection conditions were also studied. Using Eu(III) as a fluorescence probe, which was excited at 241 nm, the decreased fluorescence intensity at 485 nm of the system (ΔF) showed a good linear relationship with the concentration of prulifloxacin within the range 5.0×10^{-8}-1.00×10^{-5} mol L^{-1} and the regression equation was $\Delta F = -6.54 + 3.53 \times 10^{-6}$ C(mol L^{-1}) (n = 7). The correlation coefficient was 0.9989 and the detection limit (3 σ/k) for the determination of prulifloxacin was 2.8×10^{-8} mol L^{-1}. This method is simple, practical and relatively free interference from coexisting substances and can be successfully applied to determination of prulifloxacin.

Key Words: Eu(III), Prulifloxacin, Spectrofluorimetry, Fluorescence intensity.

INTRODUCTION

Many new fluoroquinolone derivatives, such as grepafloxacin[1], ciprofloxacin[2] and pazufloxacin[3], have subsequently been developed. They possess even greater activity against gram-positive bacteria and anaerobes, and thus they are now widely used for clinical treatment. Prulifloxacin (PUFX), a new and lipophilic prodrug of ulifloxacin[4], is a broad-spectrum oral fluoroquinolone antibacterial agent[5], characterized by a potent and broad-spectrum antibacterial activity *in vitro* activity against various gram-negative and gram-positive bacteria and several anaerobic and atypical bacteria commonly associated with chronic bronchitis ,urinary infections and respiratory tract infections[6,7]. Prulifloxacin (Fig. 1), 6-fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1, 3-dioxolen-4-yl)-methy-1-piprazinyl]-4-oxo-4*H*-[1,3]thiaceto[3,2-α]quinolone-3-carboxylic acid, is a fourth-generation member of the synthetic fluoroquinolone group of antibiotics, which exhibits a greater intrinsic antibacterial activity and a broader antibacterial spectrum than other antibiotics. It is a new thiazeto-quinolone antibacterial agent which acts directly on bacterial DNA gyrase inhibiting cell reproduction that leads to cell death[8]. Prulifloxacin structure contains the skeletal quinolone with a four-member ring in the 1,2-position including a sulfur atom to increase antibacterial activity and an oxodioxolenylmethyl group in the 7-piperazine ring to improve its oral absorption. After prulifloxacin is oral administrated, it is absorbed by the intestine and enters the circulation, where it is immediately

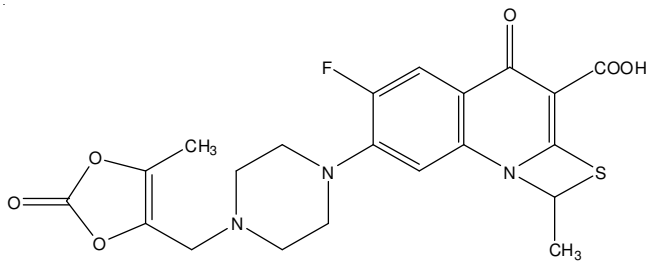

Fig. 1. Structure of prulifloxacin

and quantitatively transformed[9] into its active metabolite AF 3013, other metabolites accounting for < 15 % of the administered dose[10].

Several analytical techniques have been used for the determination of prulifloxacin, such as high-performance liquid chromatography (HPLC) coupled with UV detection[11,12], fluorescence detection[13] and mass spectrometry[14]. The HPLC method was characterized by good sensitivity, accuracy and precision, but the sample processing was relatively complicated, the time was relatively longer, the analytical conditions were harsh and the equipment was expensive. Therefore, a simple, direct, rapid, sensitive and reliable analytical method was demanded.

The spectrofluorimetric method has been widely used to estimate pharmaceuticals[15,16] owing to its simplicity, rapidity, high sensitivity and selectivity. Fluorescence features of lanthanide ions [especially Tb(III)and Eu(III)] allows their application as the fluorescence probes.

The present paper describes a rapid, selective and sensitive spectrofluorimetric method using the Eu(III) as the fluorescence probes for the determination of prulifloxacin. Optimum conditions for the determination of prulifloxacin were studied through branch experiments in this paper. The described method is validated in terms of selectivity, sensitivity, linearity, accuracy, precision and stability.

EXPERIMENTAL

Absorption spectra were recorded on a TU1901 UV-VIS spectrophotometer (PGeneral, Beijing, China). The Cary Eclipse fluorescence spectrophotometer (Varian, America) was applied to record the fluorescence spectra. The pH values were measured with a PHS-3C meter (Shanghai Lida Instrumentation Co. Ltd., China).

Stock standard solution of prulifloxacin (1.0×10^{-4} mol L^{-1}) (99.0 % purity) was prepared by dissolving 0.0116 g of prulifloxacin (461.46 Da, Shanghai Modern Pharmaceutical Co. Ltd. Shanghai, PR China) in 1.0 mL 0.20 mol L^{-1} HCl and diluting with double-distilled water to 250 mL. Working prulifloxacin solutions were prepared by diluting the stock solution with double distilled water daily.

A stock solution of $EuCl_3$ (1.8×10^{-3} mol L^{-1}) was prepared by dissolving 0.0634 g Eu_3O_4 (99.0 % purity, Sinopharm Chemical Reagent Co. Ltd., China) in warm HCl and evaporating the solution to near dryness before diluting to 100 mL with water and diluted to the desired concentration when used. The working Eu(III) solution was obtained by appropriate dilution of the stock solution with double-distilled water Britton-Robinson buffer solutions (B-R) were prepared by mixing the mixed acid (composed of 0.04 mol L^{-1} H_3PO_4, CH_3COOH and H_3BO_3) with 0.2 mol L^{-1} NaOH in proportion. The buffer solutions were prepared to adjust the acidity of the system. The pH value of solutions was kept at 8.95.

Double-distilled water made in-house was used throughout. All chemicals used were of analytical-reagent or higher grade.

Preparation of samples: Prulifloxacin (2.5×10^{-6} mol L^{-1}) and prulifloxacin (3.0×10^{-6} mol L^{-1}) were prepared by diluting the stock standard solution of prulifloxacin with double-distilled water.

General procedure: All studies were carried out in 10 mL calibrated tubes. In tubes, 1 mL known B-R buffer solution, 1.0 mL of 1.8×10^{-4} mol L^{-1} Eu(III) solution and a known volume of the prulifloxacin solution were added. Then the solution was diluted to 10 mL with water and mixed well. After reaction for 0.5 h, the solutions were taken into the optical cell. The fluorescence spectra of the system were recorded on a Cary Eclipse fluorescence spectrometer at 300-600 nm. Both the excitation and emission slit for all fluorescence measurements were maintained at 5 nm. The fluorescence intensity was measured with a 1 cm quartz cell by an excitation wavelength of 241 nm and an emission wavelength of 485 nm. The decreased fluorescence intensity was represented as $\Delta F = F_0 - F$, where F and F_0 were the fluorescence intensities of the systems with and without prulifloxacin. The ultraviolet (UV) absorption spectra were measured on a TU1901 UV-VIS spectrophotometer. The absorbances were measured at 250-300 nm. The pH values were measured with a PHS-3C meter.

RESULTS AND DISCUSSION

Fluorescence spectra: The fluorescence emission spectra of Eu(III) (1.8×10^{-5} mol L^{-1}) (1), prulifloxacin (1.0×10^{-6} mol L^{-1}) (2), Eu(III) (1.8×10^{-5} mol L^{-1}) + prulifloxacin (1.0×10^{-6} mol L^{-1}) (3) systems by an excitation wavelength of 275 nm are shown in Fig. 2. Both the excitation and emission slit were at 5 nm. As shown in Fig. 2, Eu(III)-solution and prulifloxacin exhibit the strong fluorescence emission band at 552 and 416 nm, respectively. When added Eu(III) into the prulifloxacin system, the fluorescence intensity of the prulifloxacin was enhanced and the fluorescence intensity of the Eu(III) was decreased remarkably. It indicates that the prulifloxacin molecule and Eu(III) ion could undergo a radiationless energy transfer reaction.

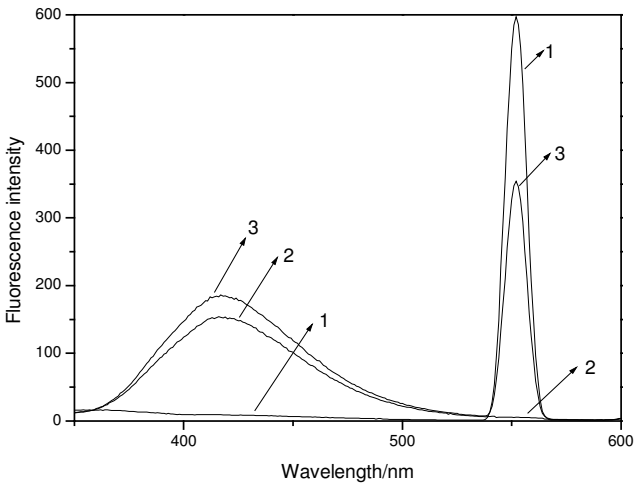

Fig. 2. Fluorescence emission spectra. λ_{ex} = 275 nm. T = 290 K, pH = 8.95, 1. Eu(III) (1.8×10^{-5} mol L^{-1}), 2. prulifloxacin (1.0×10^{-6} mol L^{-1}), 3. Eu(III) (1.8×10^{-5} mol L^{-1}) + prulifloxacin (1.0×10^{-6} mol L^{-1})

Absorption spectra: The ultraviolet (UV) absorption spectra of Eu(III), Eu(III) + B-R, prulifloxacin, prulifloxacin + B-R, Eu(III) + prulifloxacin + B-R are shown in Fig. 3 Curve 1 and curve 2 indicate that Eu(III) has no absorbance in the 250-300 nm ranges. From curve 3 we know that prulifloxacin has a strong absorbance at 271 nm, curve 4 shows that the absorbance of prulifloxacin slightly increased when the B-R was added. Comparing curve 4 with curve 5, after the addition of Eu(III) ion into the prulifloxacin solution, the absorbance of prulifloxacin increased, which indicates that prulifloxacin may form a binary complex with Eu(III) ion.

Influence factors on the fluorescence intensity of the system

Effect of Eu(III) ion concentration: When the concentration of prulifloxacin was fixed at 1.0×10^{-6} mol L^{-1}, the effect of Eu(III) concentration on the fluorescence intensity of the system was studied. As shown in Fig. 4, the fluorescence emission spectra of prulifloxacin with various amounts of Eu(III) following an excitation at 275 nm. prulifloxacin exhibits a strong fluorescence emission band at 416 nm. The fluorescence intensity enhanced gradually with the increase in concentration of Eu(III). The fluorescence intensity reached the maximum when the concentration of Eu(III) was 1.8×10^{-5} mol L^{-1} and the fluorescence intensity remained constant when

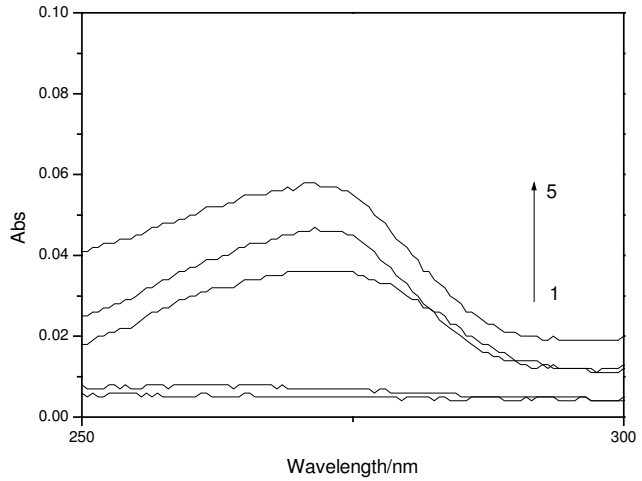

Fig. 3. Ultraviolet (UV) absorption spectra. T = 290 K, pH = 8.95 $C_{prulifloxacin}$ = 1.0 × 10^{-6} mol L^{-1}, $C_{Eu^{3+}}$ =1.8 × 10^{-5} mol L^{-1}. 1. Eu(III), 2. Eu(III) + B-R, 3. prulifloxacin, 4. prulifloxacin + B-R, 5. Eu(III) + prulifloxacin + B-R

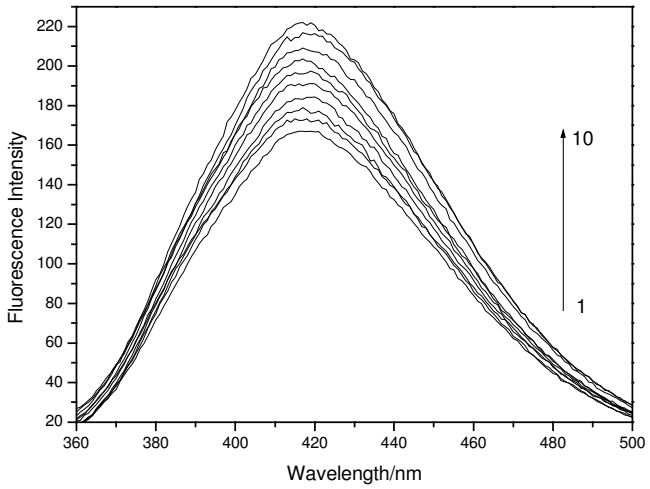

Fig. 4. Effect of Eu(III) ion concentration, λ_{ex} = 275 nm, T = 290 K, pH = 8.95, $C_{prulifloxacin}$ = 1.0 × 10^{-6} mol L^{-1}, the concentrations of Eu(III) from 1-10 were as follows: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 (1.0 × 10^{-5} mol L^{-1})

the concentration of Eu(III) was continued increasing. So 1.8 × 10^{-5} mol L^{-1} was selected as the Eu(III) concentration for further research.

Effect of pH and buffers: The changes of pH would influence the compositions and stabilities of the fluorescent complexes and resulted in changes in the fluorescence characters. So a suitable pH was important for the fluorescence character of the complexes. Effect of pH on the fluorescence intensity of the system was studied. As shown in Fig. 5, it could be seen that ΔF decreased with an increase in pH when pH was lower than 8.95, reached maximum when pH was 8.95 and then sharply decreased when pH was above 8.95. Hence, a pH of 8.95 was selected for further research.

The experiments indicated that the buffers also had a large effect on the fluorescence intensity of the system. Some buffers were tested as follows: KH_2PO_4, HMA, NH_4Cl-NH_3 and B-R buffer solutions. From the experimental results, it is found that B-R buffer solution was the most suitable buffer.

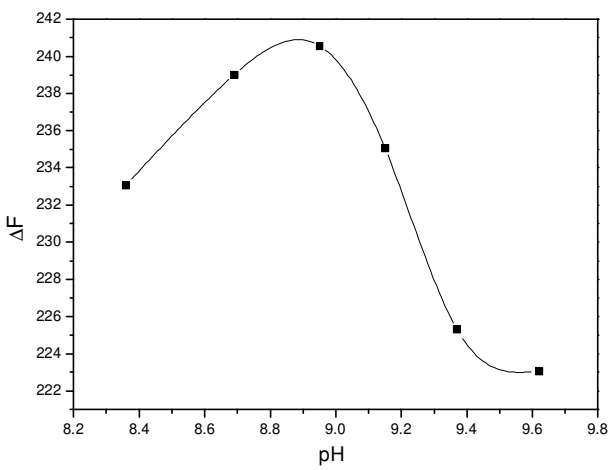

Fig. 5. Effect of pH on the fluorescence intensity of the system, T = 290 K, $C_{prulifloxacin}$ = 1.0 × 10^{-6} mol L^{-1}, $C_{Eu^{3+}}$ = 1.8 × 10^{-5} mol L^{-1}

Effect of buffer solution content: Effect of buffer solution content on the fluorescence intensity of the system was investigated in this study, where the maximum decreased fluorescence intensity of the system (ΔF) was observed from Fig. 6 at 1 mL buffer solution. Thus, 1 mL B-R buffer solution was chosen as the most suitable for further study.

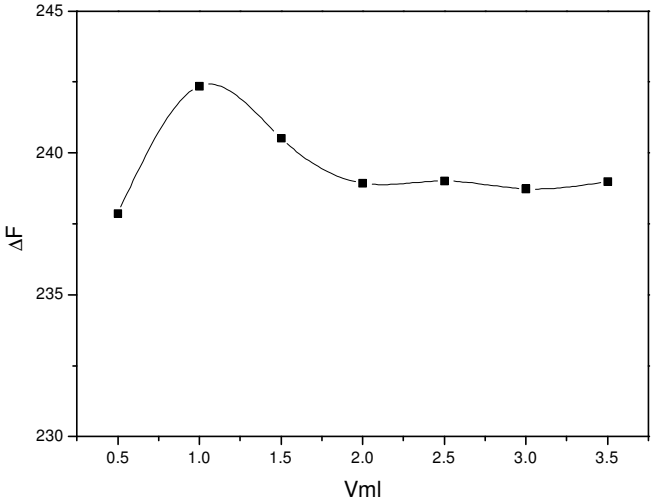

Fig. 6. Effect of buffer solution content. T = 290 K, pH = 8.95, $C_{prulifloxacin}$ = 1.0 × 10^{-6} mol L^{-1}, $C_{Eu(III)}$ = 1.8 × 10^{-5} mol L^{-1}

Effect of reaction time: The reaction of the prulifloxacin-Eu(III) system could complete in about 0.5 h at room temperature. The fluorescence intensity reached its highest value and remained constant for at least 2 h. The fluorescence of the system in 10-120 min has been estimated and the results are shown in Fig. 7. Hence, all measurements were made at 0.5 h after all reagents were added in further study.

Effect of the addition order of reagents: The fluorescence of a series of solutions with different addition orders of same concentrations of reagents (F) and their corresponding blank solutions (F_0) were measured at $\lambda_{ex}/\lambda_{em}$ = 241 nm/485 nm adding the reagents in different orders had a negligible influence on the F, F_0 and ΔF values. Considering the stability of the system, along with the F, F_0 and ΔF, the following order was optimal: Eu(III), prulifloxacin, buffer solution.

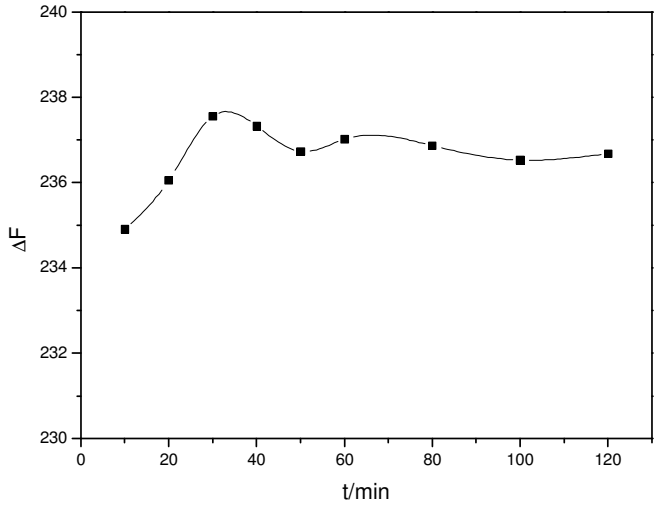

Fig. 7. Effect of reaction time. T = 290 K, pH = 8.95, $C_{prulifloxacin}$ = 1.0 × 10^{-6} mol L^{-1}, $C_{Eu(III)}$ = 1.8 × 10^{-5} mol L^{-1}

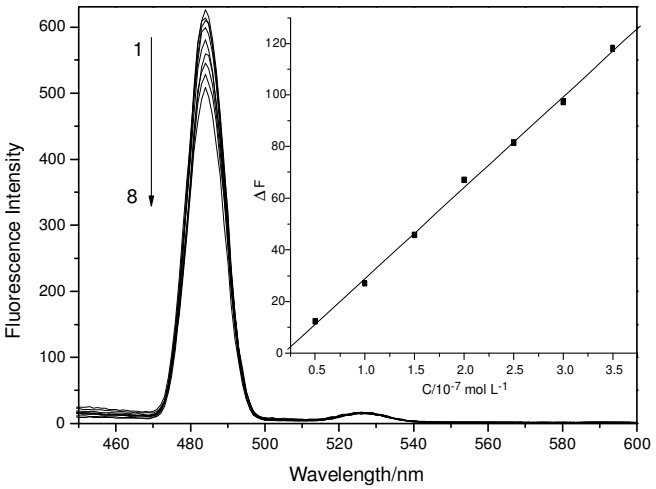

Fig. 8. Calibration curve graphs for the determination of prulifloxacin, T = 290 K, pH = 8.95, λ_{ex} = 241 nm, $C_{Eu(III)}$ = 1.8 × 10^{-5} mol L^{-1}, the concentrations of prulifloxacin from 1-8 were as follows: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 (1.0 × 10^{-7} mol L^{-1})

Interferences of foreign substances: Under the optimum conditions, effects of interferences that usually used as the compatibility of medicines on the fluorescence intensity of the system were studied. At the prulifloxacin concentration of 1.0 × 10^{-6} mol L^{-1}, the highest permissible molar excesses of other substances causing a ± 5 % relative error in the fluorescence intensity were investigated. As shown in Table-1, no significant interference could be observed for these foreign substances.

TABLE-1
INFLUENCE OF COEXISTING SUBSTANCE ON PRULIFLOXACIN-Eu(III) SYSTEM

Coexisting substance	Concentration (mol L^{-1})	ΔF (%)
Pepsin	1.0 × 10^{-6}	-4.6
Ribonucleic acid (yeast)	1.0 × 10^{-5}	4.2
L-Histidine	1.0 × 10^{-4}	0.1
Glucose	1.8 × 10^{-4}	3.7
Starch	2.5 × 10^{-3}	-2.8
Ca^{2+}	0.4 × 10^{-4}	2.1
Cu^{2+}	0.6 × 10^{-4}	2.2
Zn^{2+}	1.0 × 10^{-4}	1.8
Mg^{2+}	1.0 × 10^{-4}	1.8
Al^{3+}	1.0 × 10^{-4}	3.9

Analytical application

Calibration curve and detection limit: Under optimal conditions described above, calibration graphs for the determination of prulifloxacin were constructed. Both the excitation and emission slit were maintained at 5 nm. The fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 241 nm/485 nm with a 1 cm quartz cell. It is shown in Fig. 8, the decreased fluorescence intensity of the prulifloxacin-Eu(III) system showed a good linear relationship with the concentration of prulifloxacin in the range of 5.0 × 10^{-8}-1.0 × 10^{-5} mol L^{-1} and the regression equation was ΔF = -6.54 + 3.53 × 10^{-6} C (mol L^{-1}) (n = 7), correlation coefficient was 0.9989. The detection limit (3 σ/k) for the determination of prulifloxacin was 2.8 × 10^{-8} mol L^{-1} and relative standard deviation was 3.2 % for the determination of 4.0 × 10^{-7} mol L^{-1} prulifloxacin (n = 11).

Analysis of samples: The proposed method was applied to determine prulifloxacin in the samples prepared above and the results obtained are given in Table-2. The recoveries of prulifloxacin in the sample 1 and sample 2 were 102.8 and 99.4 %, respectively.

Conclusion

Prulifloxacin is a kind of antibiotics drug containing α-carbonyl carboxylic acid configuration. A literature survey[17] showed that the α-carbonyl carboxylic acid moiety allows efficient energy transfer from Eu(III) ion to the ligands with a high fluorescence quantum yield, large Stokes' shift, narrow emission bonds and large fluorescence lifetime; hence, it avoids potential background fluorescent emission interference from the biological matrix[18].

In this paper, using the Eu(III) as the fluorescence probes for the determination of prulifloxacin, as for the complex of Eu(III)-prulifloxacin, the fluorescence intensity of the prulifloxacin was enhanced and the fluorescence intensity of the Eu(III) was decreased remarkably through the intramolecular energy transfer process.

Compared with the reported HPLC method, the present spectrofluorimetric method can directly determine prulifloxacin with sufficient selectivity, acceptable accuracy and precision and satisfactory recovery as well as rapidity. Through the above experiments, we found the optimum reaction conditions for

TABLE-2
RESULTS OF THE DETERMINATION OF PRULIFLOXACIN SAMPLES

Prulifloxacin samples	Prulifloxacin samples (10^{-6} mol L^{-1})	Found		Average recovery (%)	RSD (%)
		Single found (10^{-6} mol L^{-1})	Average (10^{-6} mol L^{-1})		
1	2.5	2.64, 2.65, 2.57, 2.45, 2.53	2.57	102.8	3.2
2	3.0	3.09, 2.86, 2.93, 3.12, 2.90	2.98	99.4	3.4

the determination of prulifloxacin, the pH 8.95, 0.5 h and 1 mL were selected as the optimum value of the acidic medium, the optimum reaction time and the content of B-R buffer solution, respectively. The results of this study shows that using the Eu(III) as the fluorescence probes for the determination of prulifloxacin is feasible and get satisfactory results.

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