Rapid europium-sensitized fluorescent determination of ulifloxacin, the active metabolite of prulifloxacin, in human serum and urine

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Abstract: A new fluorescent method was developed based on the ulifloxacin-europium (III)-sodium dodecylbenzene sulfonate system for the determination of ulifloxacin, the active metabolite of prulifloxacin. Sodium dodecylbenzene sulfonate formed a ternary complex with ulifloxacin-europium (III) and significantly enhanced the characteristic fluorescence of europium (III). The enhanced fluorescence intensity showed a good linear relationship with the concentration of ulifloxacin in the range of 5.0 × 10⁻⁸ - 2.0 × 10⁻⁶ M with a detection limit of 2.0 × 10⁻¹⁰ M (3σ). This method is rapid and sensitive, and has been successfully applied to the determination of ulifloxacin in human urine and serum samples.

Keywords: ulifloxacin; prulifloxacin; europium; fluorescence; sodium dodecylbenzene sulfonate

1 Introduction

Prulifloxacin (PUFX, NM441, Figure 1), the prodrug of ulifloxacin (UFX, NM394, Figure 1), is a broad-spectrum oral fluoroquinolone antibacterial agent [1]. After oral administration and intestinal absorption, PUFX is rapidly metabolized by paraoxonases into UFX, the active metabolite of prulifloxacin [2-7]. Therefore, the in vitro antimicrobial activity was studied using ulifloxacin [6, 8,9].

![Chemical structures of ulifloxacin (UFX) and prulifloxacin (PUFX)](image)

For the pharmacokinetic study of a prulifloxacin formulation product in healthy volunteers, a rapid, selective and sensitive analytical method is required to accurately determine the ulifloxacin concentration in body fluid. In previous studies, analysis of ulifloxacin has been performed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [10], HPLC with fluorescence detection [11], HPLC-MS/MS [12], capillary zone electrophoresis [13], flow-injection chemiluminescence [14] and terbium-sensitized second-order scattering spectrofluorimetry [15].

Europium-sensitized fluorescent determination is an indirect method involving complexation of ligand molecules and europium ions. The intramolecular energy transfer from the absorbing ligand to the europium ions results in the emission of a strong narrow-band fluorescence with a large Stokes shift and long decay time. As a result, it could avoid the potential background fluorescent interferences from the biological matrix [16]. Quinolones and fluoroquinolones have suitable functional groups (carboxylic and keto-oxygen atoms) to form stable complexes with lanthanide ions. This technique has been widely utilized for the determination of quinolones norfloxacin [17], enoxacin [18], garenoxacin [19], ciprofloxacin [20], and trovafloxacin [21]. But the sensitivity is not very high as all the detection limits of these methods are over the range of 1.2 × 10⁻⁹ to 4.7 × 10⁻⁸ M. However, the europium-sensitized fluorescent determination method described here could provide a much higher sensitivity. The detection limit (3σ) could attain 2.0 × 10⁻¹⁰ M. Furthermore, there are few reports about the fluorescence mechanism. Compared with most of other approaches for the determination of UFX, the proposed method offers advantages in higher sensitivity, wider linear range, and better stability, which has been successfully applied to the determination of ulifloxacin in human urine and serum samples with satisfactory results, and the fluorescence enhancement mechanism is also discussed. To our knowledge, this is the first time that the europium-sensitized fluorescent determination is utilized for the determination of UFX.

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2 Materials and methods

2.1 Apparatus

Fluorescence intensity was measured on an Hitachi F-2500 fluorescence spectrophotometer (Japan), using a standard 10 mm path-length quartz cell, with 10 nm bandwidths for both the excitation and emission monochromators. Absorption spectra were measured on an UV-2401PC spectrophotometer (Shimadzu, Japan) equipped with 10 mm path-length quartz cells. The surface tension was measured on a Kruss tensiometer (Shanghai, China). Serum samples were centrifuged 800 × g for 5 min at 1500 × g [19]. No sample pre-treatment was required for urine samples. Further dilution with water was made to ensure that the concentrations of the drug in the sample solutions fall within the linear range of the method.

3 Results and discussion

3.1 Spectral characteristics

The excitation and emission spectra of UFX (1, 1'), Eu³⁺ (2, 2'), UFX-Eu³⁺ (3, 3'), and UFX-Eu³⁺-SDBS (4, 4') system are shown in Figure 2. The excitation spectrum of the UFX-Eu³⁺-SDBS system exhibited a peak at 276 nm, which was selected as the excitation wavelength. From the emission spectra, no characteristic fluorescence of Eu³⁺ was observed in the Eu³⁺ system (2'), while weak characteristic fluorescence of Eu³⁺ was observed in the UFX-Eu³⁺ system (3'). Curve 4' is the excitation spectrum of UFX-Eu³⁺-SDBS system and strong emission peaks were observed at 594 and 616 nm, corresponding to transitions of the Eu³⁺-D₃⁻F₁ and D₅⁻F₂, respectively. Compared with the UFX-Eu³⁺ system, the fluorescence intensity (Iₑ) was enhanced about 37-fold by the addition of SDBS. Thereby, 276 nm and 616 nm were chosen as the excitation wavelength and emission wavelength, respectively.
3.2 Effects of pH and buffers

The effect of pH on the fluorescence intensity of the system was studied in the range of 5.8–10.5. The results indicated that the optimal pH value was in the range of 8.2–8.8 (Figure 4), so pH 8.6 was selected for the recommended procedure. Setting pH at 8.6, the effect of the following buffer solutions on the fluorescence intensity was then examined, including NH₄Ac-NH₃·H₂O, NaAc-HAc, NH₄Cl-NH₃·H₂O, Tris-HCl, and KH₂PO₄-NaOH. It was found that UFX-Eu³⁺-SDBS fluorescence system in the NH₄Cl-NH₃·H₂O medium of the concentration 0.01 M gave the highest sensitivity. Therefore, a 0.01 M NH₄Cl-NH₃·H₂O buffer solution of pH 8.6 was chosen for further study.

3.3 Effect of europium (III) concentration

With a fixed UFX concentration of 5.0×10⁻⁶ M, the effect of Eu³⁺ concentration on the fluorescence intensity of the system was investigated (Figure 5). Fluorescence intensity reached a maximum value with a Eu³⁺ concentration of 5.0×10⁻⁶ M. Therefore, the Eu³⁺ concentration of 5.0×10⁻⁶ M was selected for further experiments. ∆Fl was the net fluorescence intensity, with the background signal being subtracted.

In order to investigate the effect of micellar media on the fluorescence intensity, various types of surfactants including CTAB (Cetrimonium bromide, cationic), GA (Arabic gum, non-ionic), OP (Polyoxyethylene nonylphenol ether, non-ionic), β-CD (β-cyclodextrin, non-ionic), Triton X-100 (non-ionic), Pluronic F68 (non-ionic), PEG40 (non-ionic), SDS (Sodium dodecyl sulfate, anionic), and SDBS (anionic) were studied (Table 1). It can be seen that there was no significant fluorescence enhancement in the presence of cationic surfactants, non-ionic surfactants or anionic surfactant SDS. However, a significant increase in fluorescence was observed in the presence of anionic surfactant SDBS. The effect of the SDBS concentration on the fluorescence intensity was also investigated (Figure 6). Maximum fluorescence intensity was observed at an SDBS concentration of 1.0×10⁻⁴ M, which was chosen for further experiments.

The mechanism of SDBS effect was studied by investigating the changes of surface tension of the fluorescence system with an increase of the SDBS concentration (Figure 7). It can be seen that the surface tension first decreased sharply, and then reached equilibrium at the SDBS concentration of 7.0×10⁻⁴ M, which can be considered as the apparent critical micelle concentration (CMC) of SDBS in this system. Since the SDBS concentration selected for the study (1.0×10⁻⁴ M) was well above the CMC, it could be concluded that the formation of micelles had a great impact on the enhancement of the fluorescence intensity of the system.
Table 1  Effects of different surfactants

| Surfactants                     | Optimal concentration | Relative fluorescence intensity (%) |
|---------------------------------|-----------------------|-----------------------------------|
| Cetrimonium bromide (CTAB)      | 3.0×10⁻¹⁴M           | 6.2                               |
| β-cyclodextrin (β-CD)           | 2.0×10⁻¹⁴M           | 0                                 |
| Polyoxethylene nonylphenol ether (OP) | 10% (v/v)   | 0                                 |
| Triton X-100                    | 10% (v/v)            | 0                                 |
| Arabic gum (GA)                 | 3.0 g/L              | 3.8                               |
| Pluronic F68                    | 2.5 g/L              | 2.7                               |
| PEG40                           | 3.0 g/L              | 5.4                               |
| Sodium dodecyl sulfate (SDS)    | 1.0×10⁻¹⁴M           | 4.7                               |
| Sodium dodecylbenzene sulfonate (SDBS) | 1.0×10⁻¹⁴M       | 100.0                             |

Table 2  Maximum permissible concentration of interference species

| Interference species | Maximum permissible concentration (M) | Change of ΔI (% |
|----------------------|---------------------------------------|----------------|
| Fe³⁺ , Cl⁻           | 1.0×10⁻⁷                          | 5.0             |
| Cu²⁺ , SO₄²⁻           | 4.0×10⁻⁷                          | 4.1             |
| Vitamin C             | 1.5×10⁻⁵                          | -4.8            |
| Zn²⁺ , SO₄²⁻           | 5.0×10⁻⁴                          | -4.6            |
| L-Methionine          | 2.0×10⁻⁵                          | -4.7            |
| As³⁻ , Cl⁻            | 5.0×10⁻³                          | 5.0             |
| L-Histidine           | 1.0×10⁻⁴                          | -4.6            |
| NH₄⁺ , Cl⁻            | 1.5×10⁻⁴                          | -4.4            |
| Mg²⁺ , Cl⁻            | 2.0×10⁻⁴                          | 4.5             |
| Ca²⁺ , Cl⁻            | 3.0×10⁻⁴                          | 4.2             |
| L-Glutamine           | 4.0×10⁻⁴                          | -4.8            |
| L-Cysteine            | 1.0×10⁻³                          | 4.3             |
| Glycine               | 1.0×10⁻³                          | -4.2            |
| Glucose               | 1.5×10⁻³                          | -4.5            |
| Na⁺ , Cl⁻             | 1.0×10⁻²                          | 4.4             |
| K⁺ , Cl⁻              | 1.0×10⁻²                          | 4.6             |

3.6 Effect of interfering species

The interference of metal ions, glucose, and amino acids which are commonly presented in vivo was studied by adding different amounts of these compounds to a 5.0×10⁻⁶ M UFX solution. The maximum permissible concentrations of the interfering substances causing a ±5% relative error in the fluorescence intensity are shown in Table 2. The results indicated that most species except for Cu²⁺ and Fe³⁺ had little effect on the fluorescence intensity of the system.

3.7 Analytical application

3.7.1 Linear range and detection limit

Under the optimal experimental conditions, the enhanced fluorescence intensity of the system (ΔIₓ) responded linearly to the UFX concentration in the range of 5.0×10⁻⁸ - 2.0×10⁻⁶ M with a correlation coefficient of 0.9990. The detection limit for UFX calculated from the standard deviation of the blank (the reagent blank without UFX, n = 20) (3σ) is 2.0×10⁻¹⁰ M.

In comparison with other methods reported, the rapid and simple method proposed in this paper offers significantly increased sensitivity (Table 3).
diluted 1,000-fold with water and then analyzed by standard addition method, and serum samples were deproteinated first and then diluted 40-fold prior to analysis. The results of spiked urine and serum samples are listed in Table 4.

| Method                                  | Detection limit (M) | Linear range (M) | Application                          | References |
|-----------------------------------------|---------------------|------------------|--------------------------------------|------------|
| HPLC                                    | 1.7 × 10^{-8}       | 1.4 × 10^{-6} - 1.7 × 10^{-6} | Aqueous human humor                  | [10]       |
| HPLC-Fl                                 | 2.9 × 10^{-8}       | 2.9 × 10^{-6} - 2.9 × 10^{-6} | Human plasma                         | [11]       |
| HPLC-MS                                 | 7.2 × 10^{-9}       | 1.4 × 10^{-8} - 1.4 × 10^{-5} | Human plasma                         | [12]       |
| Capillary zone electrophoresis          | 5.7 × 10^{-8}       | 5.7 × 10^{-6} - 5.7 × 10^{-6} | Human plasma                         | [13]       |
| Chemiluminescence                       | 5.5 × 10^{-8}       | 1.0 × 10^{-8} - 5.0 × 10^{-6} | Spiked human serum and urine          | [14]       |
| Terbium-sensitized second-order scattering spectrofluorimetry | 3.9 × 10^{-8} | 2.0 × 10^{-8} - 1.0 × 10^{-5} | Spiked human serum and urine          | [15]       |
| Europium probe spectrofluorimetry       | 2.0 × 10^{-10}      | 5.0 × 10^{-8} - 2.0 × 10^{-6} | Spiked human serum and urine          | Present method |

| Table 4 Recovery of UF in spiked human urine and serum samples (n = 5) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sample          | Added (M)       | Found (M)       | Recovery ± RSD (%) |
| Urine 1         | 2.5 × 10^{-7}   | 2.6 × 10^{-7}   | 104.0 ± 0.1     |
| Urine 2         | 3.8 × 10^{-7}   | 3.8 × 10^{-7}   | 100.0 ± 2.7     |
| Serum 1         | 2.0 × 10^{-7}   | 2.1 × 10^{-7}   | 105.0 ± 3.0     |
| Serum 2         | 3.0 × 10^{-7}   | 3.0 × 10^{-7}   | 100.0 ± 2.3     |
| Serum 3         | 4.0 × 10^{-7}   | 3.9 × 10^{-7}   | 97.5 ± 2.9      |

4 Conclusion

A new europium-sensitized fluorescence method for the determination of UF is proposed here. Under optimal conditions, the enhanced fluorescence intensity is in proportion to the UF concentration over the range of 5.0 × 10^{-8} - 2.0 × 10^{-6} M with a detection limit (3σ) of 2.0 × 10^{-7} M. In comparison with most of methods reported for the determination of UF, the proposed method is faster and simpler, and has higher sensitivity. It has been successfully applied for the determination of UF in human urine and serum samples with minimal sample pretreatment. The research on fluorescence mechanism reveals that the formation of micelles plays an important role in the fluorescence enhancement in the complex system studied.

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