Talanta 82 (2010) 1858–1863

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Synchronous fluorescence measurement of enrofloxacin in the pharmaceutical formulation and its residue in milks based on the yttrium (III)-perturbed luminescence

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ARTICLE INFO

Article history:
Received 17 June 2010
Received in revised form 28 July 2010
Accepted 31 July 2010
Available online 8 August 2010

Keywords:
Enrofloxacin
Fluoroquinolones
Synchronous fluorescence
Residue
Milk
Determination

ABSTRACT

A simple, rapid and sensitive synchronous fluorescence method is put forward for the determination of enrofloxacin (ENRO) in the pharmaceutical formulation and its residue in milk based on the yttrium (III)-perturbed luminescence. When Y^{3+} is added into the ENRO solution, the fluorescence of ENRO is significantly enhanced. The synchronous fluorescence technology is employed in the method to determine trace amount of ENRO residue in milks. The synchronous fluorescence intensity of the system is measured in a 1-cm quartz cell with excitation wavelength of 328 nm, $\Delta\lambda = 80$ nm. A good linear relationship between the fluorescence intensity and the ENRO concentration is obtained in the range of 1.0×10^{-9} to 2.0×10^{-6} mol L^{-1} ($r^2 = 0.9992$). The limit of detection (LOD) of this method attains as low as 3.0×10^{-10} mol L^{-1} (S/N = 3). The selectivity of this method is also very good. Common metal ions, rare-earth ions and some pharmaceuticals, which are usually used together with ENRO, do not interfere with the determination of ENRO under the actual conditions. The proposed method can be applied to determine ENRO residue in milks, and limit of quantification (LOQ) determined in the spiked milk is estimated to be 2.8×10^{-8} mol L^{-1} (10 μg L^{-1}). Moreover, this method can be used as a rapid screening for judging whether the ENRO residues in milks exceed Minimal Risk Levels (MRLs) or not. In addition, the mechanism of the fluorescence enhancement is also discussed in detail.

1. Introduction

Enrofloxacin [1-cyclopropyl-7-(4-ethyl-1-piperazine)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid] (ENRO, Fig. 1), belongs to the second generation of quinolone antibiotics fluorinated in position 6 and bearing a piperazinyl moiety in position 7. Enrofloxacin is the first specified fluoroquinolone developed for veterinary application. Similar to other fluoroquinolones, enrofloxacin is used in the treatment of systemic infections including urinary tract, respiratory, gastro-intestinal and skin infections. Its drug function works by inhibiting both α-subunit of bacterial DNA gyrase (topoisomerase II) and an essential enzyme that maintains the superhelical twists in the bacterial DNA, and thus affects the growth and reproduction of bacterial [1]. Because of a very broad spectrum of activity against both Gram-negative and Gram-positive bacteria and lower side effects, ENRO has been widely used for the treatment of some infectious diseases in pets and livestock.

Due to its widespread use in pets and livestock, route pharmaceutical analyses and its residual detection require simple, rapid and sensitive analytical techniques for the determination of its presence in pharmaceutical formulations and agricultural products. Several techniques have thus been developed for the determination of ENRO such as spectrophotometry [2,3], chemical luminescence [4], high performance liquid chromatography [5,6], TFC–MS/MS [7], capillary electrophoresis [8], electroanalysis [9], quantum dot [10] and immunosensor/biosensor [11,12]. However, the fluorometric method has attracted greater attention because of the combination of convenience and sensitivity [13,14]. In these fluorometric methods, metal ions especially rare-earth ions are usually used as luminescent probes to measure the pharmaceuticals [15,16]. This is because rare-earth ions (especially Tb^{3+}, Eu^{3+}) have the following luminescence characteristics: a narrow spectral width, a long luminescence life-time, a large Stokes shift and strong combination ability [17,18]. These conventional fluorometric methods are usually highly sensitive and selective for determining ENRO in pharmaceutical formulations. However, these traditional fluorometric methods cannot be applied to determine ENRO in biological fluids or agricultural products even though the deproteinization pretreatments are employed, because the proteins in biological fluids or agricultural products cannot be

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doi:10.1016/j.talanta.2010.07.082

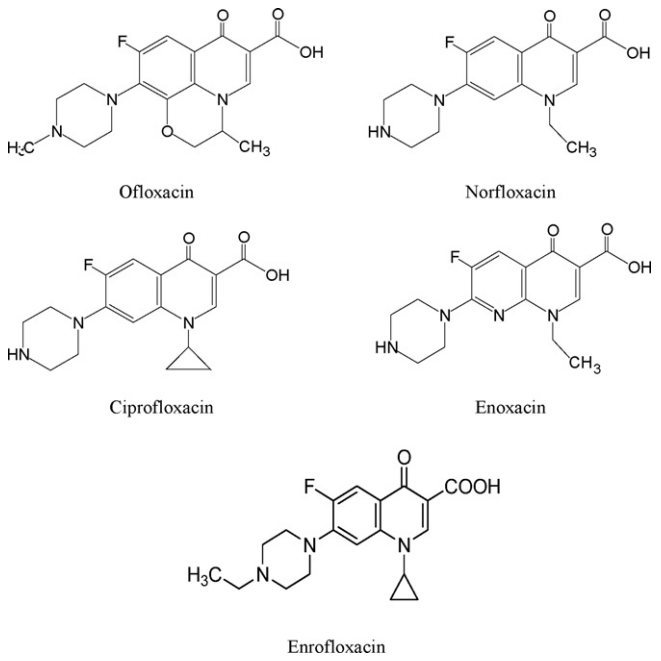

Fig. 1. Molecular structures of ofloxacin, norfloxacin, ciprofloxacin, enoxacin and enrofloxacin.

completely removed, and the residual proteins and other organic substances existing in biological fluids or agricultural products will still cause the interferences due to an overlap of the fluorescence spectra. Recently, synchronous fluorescence spectrometry (SFS) analysis has become an attractive new method for detecting drugs [19] and biomolecules [20,21]. Because SFS can provide a simplified spectrum, and it can avoid some overlapping spectra and interferences such as Rayleigh- and Raman-scattering. SFS serves as a robust method for quantitative determination in a single measurement [22].

In the present work, it is found that when Y^{3+} is added to the ENRO solution, the native fluorescence of ENRO is significantly enhanced. This new fluorescence enhancement system is different from the reported probes of metal ions or rare-earth ions (Tb^{3+}, Sc^{3+}, etc.), which are used to measure ENRO in the pharmaceuticals, and it belongs to Y^{3+}-perturbed luminescence of ligand. Furthermore, synchronous fluorescence technology is employed in this new method to eliminate the interferences from the residual proteins and other organic substances in order that this method can be applied to determine trace ENRO residues in milk. Compared with the standard method, solid-phase extraction (SPE) combined with HPLC or HPLC–MS for determining ENRO residues in milk, the proposed method does not require complicated treatment procedure such as SPE. Moreover, spectral method is usually more convenient and more rapid than HPLC method. So, this method is quite simple, rapid, and sensitive. In China, as well as in European Union (EU) and Japan, Minimal Risk Levels (MRLs) for ENRO in milk (total amount of enrofloxacin and ciprofloxacin) is 100 $\mu g\,L^{-1}$ or 100 $\mu g\,kg^{-1}$. As well, this method can be used as a simple qualitative method for rapid screening ENRO residues in milk, to judge whether its levels exceed MRLs or not. Another advantage is that the most metal ions and other pharmaceuticals usually used as the compatibility of medicines do not interfere with the determination of ENRO. This method will become a valuable tool for determining ENRO in its pharmaceutical preparations and milks, studying on pharmaceutical dynamics, and investigating its contents in the environment. In addition, a possible luminescent mechanism for this new method is also discussed in detail.

2. Experimental

2.1. Apparatus

The fluorescence spectra and intensities were acquired on a model F-2500 spectro fluorimeter (Hitachi, Japan) with a quartz cell ($1 \times 1\,cm^2$ cross-section) equipped with a xenon lamp (150 W) and a dual monochromator. All absorption spectra were recorded with a UV-2401PC spectrophotometer (Shimadzu, Japan). All pH measurements were made with a MP 220 pH meter (Mettler Toledo, China). A LD5-2A centrifuge (Beijing centrifuge machine factory, China) was used to centrifugate samples of milk.

2.2. Reagents

A stock standard solution of ENRO ($1.0 \times 10^{-3}\,mol\,L^{-1}$) was prepared by dissolving the corresponding amount of ENRO (Hangzhou Institute for drug control, China) in pure water. A stock solution of Y^{3+} ($1.0 \times 10^{-2}\,mol\,L^{-1}$) was prepared by dissolving the corresponding oxides (Y_2O_3, 99.9%, Sinopharm Chemical Reagent Co. Ltd., China) in 1:1 (v/v) hydrochloric acid and heating this solution until it is nearly dry, then diluting this solution with pure water. A $1.0\,mol\,L^{-1}$ hexamethylenetetramine (HMTA) buffer solution was prepared by dissolving the corresponding HMTA in pure water and adjusting pH to 6.3 with $1.0\,mol\,L^{-1}$ hydrochloric acid to give a final total volume of 500 mL. Acetonitrile was purchased from Tedia Company Inc. (Fairfield, USA). All chemicals were of analytical reagent grade, and pure water was obtained from a ULUPURE pure water machine (Shanghai Youpu Industry Co. Ltd., China).

2.3. Procedures

2.3.1. Milk samples preparation

Fresh milks were bought from local supermarket. ENRO residue and its metabolic product were extracted from fresh milk as follows: 1 mL fresh milk was transferred to a 10 mL centrifuge tube, then 2 mL acetonitrile was added to precipitate proteins. The mixture was vibrated for 2 min and then centrifugated at 5000 rpm for 5 min, and all the supernates were directly collected to a 10 mL colorimetric-tube for determination of ENRO residue and its metabolic product. The recovery tests in the milk were done as follows: the milk was spiked with certain concentrations of ENRO, its final concentrations in the milk were in the range of 50–200 $\mu g\,L^{-1}$, the extraction process was employed as the above procedures.

2.3.2. Synchronous fluorescence measurement

In a 10 mL colorimetric-tube, solutions were added according to the following order: 1.0 mL of ENRO standard solution with appropriate concentration, or certain volume of ENRO drug sample or extracts from milk sample, 1.0 mL of $1.0\,mol\,L^{-1}$ HMTA–HCl buffer (pH 6.3), 1.0 mL of $1.0 \times 10^{-3}\,mol\,L^{-1}$ Y^{3+} and 2 mL of acetonitrile. The mixture was diluted to 10.0 mL with pure water, shaken and allowed to stand for 10 min. The synchronous fluorescence intensity of the system was measured in a 1-cm quartz cell with excitation wavelength of 328 nm, $\Delta\lambda = 80$ nm. The excitation and emission slits for all fluorescence measurements were both maintained at 10 nm, PMT voltage is 400 V.

This method can be used as a simple qualitative method for rapid screening ENRO residues in milk, to judge whether its levels exceed MRLs or not. The detailed procedures are as follows: firstly, prepare a standard milk sample (its ENRO residues in milk are equal to 100 $\mu g\,L^{-1}$), deal with it together with other milk samples accord-

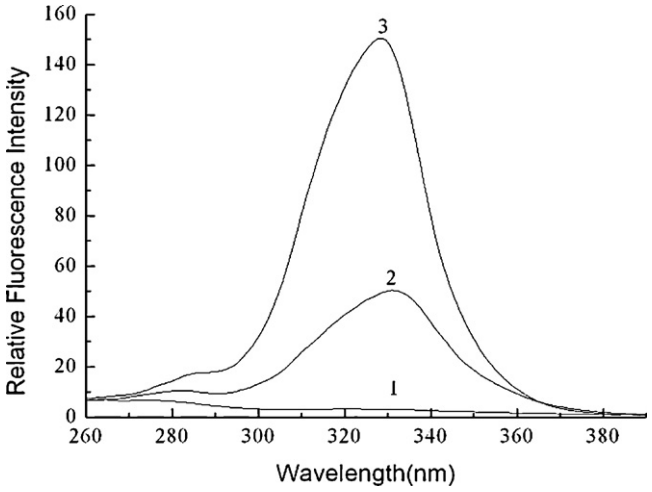

Fig. 2. Synchronous fluorescence spectra of the ENRO–Y^{3+} system ($\Delta\lambda = 80$ nm). (1) Y^{3+}; (2) ENRO; (3) ENRO–Y^{3+}. Conditions: Y^{3+}: 1.0×10^{-4} mol L^{-1}, ENRO: 1.0×10^{-7} mol L^{-1}, HMTA: 0.1 mol L^{-1}, pH = 6.3.

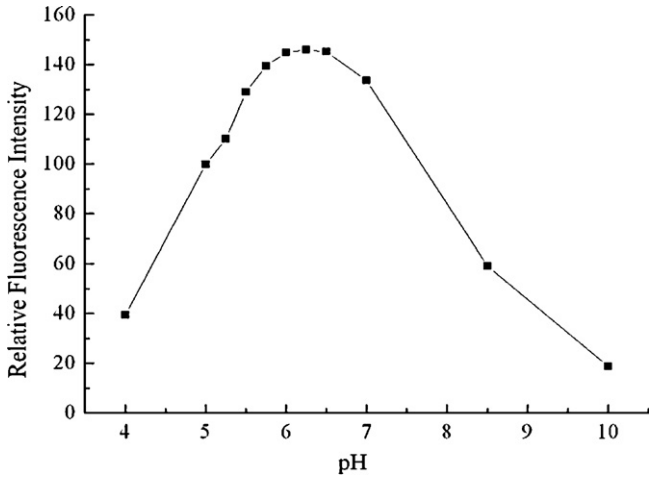

Fig. 3. Effect of pH on the fluorescence intensity of the system. Conditions: Y^{3+}: 1.0×10^{-4} mol L^{-1}, ENRO: 1.0×10^{-7} mol L^{-1}, HMTA: 0.1 mol L^{-1}.

ing to the sample preparation process; secondly, scan synchronous fluorescence spectra of these milk samples including the standard milk sample as the analytical procedures; thirdly, just comparing the fluorescence intensities of these milk samples with that of the standard milk sample. If the fluorescence intensity of certain milk sample is stronger than that of the standard milk sample, it can be primarily concluded that this milk sample might exceed MRLs. Otherwise, the result is contrary.

3. Results and discussion

3.1. Fluorescence spectra

The excitation and emission wavelengths of the native fluorescence of ENRO are 274 nm and 441 nm, respectively. The fluorescence emission peak of ENRO appeared at 424 nm in the presence of Y^{3+}, which showed a relatively strong blue-shift of 17 nm, and its fluorescence intensity was significantly enhanced. Based on the fluorescence enhancement effect of this new system, a new sensitive fluorescence method could be set up to determine ENRO. However, the application of this method was very limited except in the pharmaceutical formulation. It could not be used to determine ENRO in biological fluids or agricultural products. Although some proteins had been removed in the sample preparation process, protein residues and other organic substances existed in biological fluids or agricultural products would seriously interfere with its determination, because their fluorescence peaks almost completely overlapped. To enlarge its application in order that this sensitive method could be applied to determine ENRO residue in milk, the synchronous fluorescence technology was employed.

Fig. 2 showed that the synchronous fluorescence peak of ENRO was at 331 nm. Upon adding Y^{3+} to the ENRO solution, the fluorescence intensity of the ENRO–Y^{3+} system was significantly enhanced, which indicated that a coordination compound between ENRO and Y^{3+} ion was formed. Moreover, its emission peak showed a slight blue-shift of 3 nm. To eliminate the interferences caused by the protein residues and other organic substances in milk, the optimum $\Delta\lambda$ value was selected based on the interferences in milk and the fluorescence intensity of the system. The results indicated that when $\Delta\lambda = 80$ nm was selected, the fluorescence peak of proteins could be separated from that of ENRO. Thus, $\lambda_{ex} = 328$ nm and $\Delta\lambda = 80$ nm were selected for further study.

3.2. Optimization of the factors influencing the fluorescence intensity

3.2.1. Effects of pH and buffers

Fig. 3 showed that pH had strong effect on the fluorescence intensity of the ENRO–Y^{3+} system. The reason could be explained as follows: acidity of solution had strong influence on the formation of ENRO–Y^{3+} complex, which resulted in the strong effect on the fluorescence intensity of the system; moreover, the luminescence character of ENRO was sensitive to pH of solution, which was due to the molecular structure of ENRO with a carboxylic group. The maximum fluorescence intensity of the system was reached in the pH range of 5.8–6.5. A pH value of 6.3 was selected for further research. Under the same pH condition, the following buffers were tested for the effects on the fluorescence intensity of the system: HMTA, NaH_2PO_4–NaOH, potassium biphthalate, Britton–Robinson (BR) buffer (prepared from an acidic solution that contained 0.04 mol L^{-1} each of H_3PO_4, HAc, and H_3BO_3 by adjusting to appropriate pH using 0.2 mol L^{-1} NaOH), sodium citrate–citric acid, NaH_2PO_4–NaB_4O_7, their relative fluorescence intensities (%) were 100, 53.2, 82.6, 51.0, 55.5 and 43.7, respectively. The results showed that HMTA–HCl was the most suitable buffer, and the proper concentration of HMTA in the solution was 0.1 mol L^{-1}.

3.2.2. Effect of Y^{3+} concentration

When the concentration of ENRO was fixed at 1.0×10^{-7} mol L^{-1}, effects of Y^{3+} concentrations on the fluorescence intensities of the system were studied. Fig. 4 showed that when the concentration of Y^{3+} was lower than 1.0×10^{-4} mol L^{-1}, the fluorescence intensity of the system enhanced with the increase of Y^{3+} concentration; when the concentration of Y^{3+} increased to be 1.0×10^{-4} mol L^{-1}, the fluorescence intensity of the system reached a maximum, and with the further increase of Y^{3+} concentration the fluorescence intensity of the system still kept constant. Thus, Y^{3+} concentration of 1.0×10^{-4} mol L^{-1} was selected for further research.

3.2.3. Effect of acetonitrile

Acetonitrile as a deproteinization reagent and an organic solvent which was beneficial to extract ENRO and its metabolic product from milk, was employed in the sample preparation process. So, it was necessary that effect of acetonitrile concentration on the fluorescence intensity of the system was investigated. The results showed that the concentration of acetonitrile was less than 2% (v/v), it did not influence the fluorescence intensity of the sys-

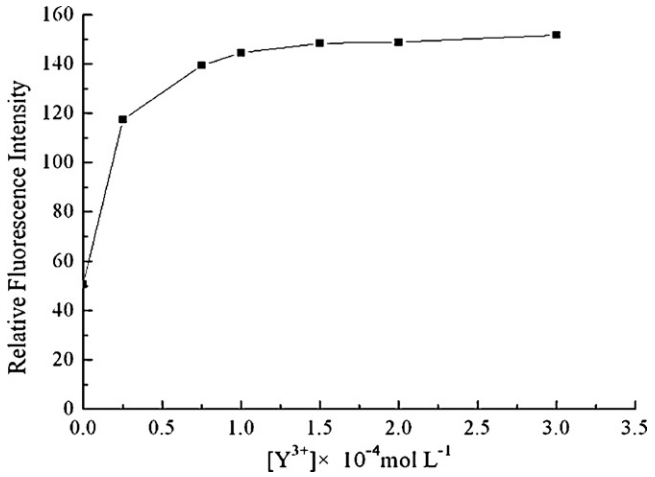

Fig. 4. Effect of Y^{3+} concentrations on the fluorescence intensity of the system. Conditions: ENRO: 1.0×10^{-7} mol L^{-1}, HMTA: 0.1 mol L^{-1}, pH = 6.3.

tem; when its concentration was between 2% and 5%, its influence on the fluorescence intensity of the system was no more than 5%; when its concentration attained 20%, it could enhance the fluorescence intensity of the system more than 10%. From the sample preparation procedures, it could be seen that the concentration of acetonitrile in the determination system could be 20%. To reduce the influence of acetonitrile on the fluorescence intensity of the system, thus in the analytical procedures, 2 mL of acetonitrile was also added to the determination system of standard solutions.

3.2.4. Stability test

The results showed that at room temperature the fluorescence intensity of the ENRO–Y^{3+} system reached a maximum in 10 min after all the reagents had been added, and remained stable for at least 3 h.

3.3. Selectivity

Under the optimal conditions, some common metal ions, rare-earth ions, and some pharmaceuticals which were possibly used together with ENRO in veterinary application, were examined for interferences. At the ENRO concentration of 1.0×10^{-7} mol L^{-1}, the highest permissible concentrations of foreign substances causing a ±10% relative error in the fluorescence intensity were listed in Table 1. Table 1 showed that, when the concentrations of the foreign substances such as metal ions, rare-earth ions and pharmaceuticals, were close to the concentration of ENRO, most of them did not interfere with the determination. In addition, four types of fluoroquinolones (FQs) were also examined for interferences. The results showed that, when the concentration ratios of ofloxacin and enoxacin to ENRO were less than of 1:1, the interferences were very small. At the same concentration, ciprofloxacin (CPFX) and norfloxacin would interfere with the determination of ENRO. Because they could also coordinate with Y^{3+}, and emitted a similar fluorescence at a wavelength identical or close to the emission peak of ENRO. Although ciprofloxacin and norfloxacin would interfere with the determination of ENRO, it did not influence its application on the determination of ENRO residues in milk. Because they belonged to the same types of FQs, they could not be used simultaneously for the treatment of some infectious diseases in pets and livestock. On the other hand, MRLs for ENRO in milk (100 μg L^{-1}) was designated "the total amount of enrofloxacin and its metabolic product (ciprofloxacin)". Thus, this point for the proposed method not only is not the shortcoming, on the contrary, this is another advantage that this method can determine the total amount of ENRO and CPFX in a single measurement.

Table 1
Effects of metal ions and other pharmaceuticals on the fluorescence intensity of the ENRO–Y^{3+} system.

Species	Concentration (mol L^{-1})	ΔIF (%)
Tb^{3+}	5.0×10^{-6}	−6.9
Dy^{3+}	5.0×10^{-7}	−8.9
La^{3+}	5.0×10^{-3}	+3.3
Eu^{3+}	1.0×10^{-5}	−6.0
Sm^{3+}	1.0×10^{-5}	−7.5
Gd^{3+}	1.0×10^{-5}	−5.4
$BaCl_2$	5.0×10^{-2}	−4.0
$FeCl_3$	5.0×10^{-6}	−6.1
$ZnSO_4$	5.0×10^{-2}	+2.8
$CaCl_2$	1.0×10^{-2}	−0.4
$MnSO_4$	5.0×10^{-4}	−7.0
NaCl	1.0×10^{-1}	−5.9
KCl	5.0×10^{-2}	−5.8
$MgSO_4$	5.0×10^{-3}	−5.8
NH_4Cl	8.0×10^{-5}	−8.5
$AlCl_3$	2.0×10^{-4}	−6.8
$CuCl_2$	1.0×10^{-6}	−5.1
Furosemide	2.0×10^{-5}	−5.6
Ranitidine hydrochloride	5.0×10^{-6}	−7.4
Fenbid	1.0×10^{-5}	−2.7
Cimetidine	2.0×10^{-5}	−9.1
Sulfamethoxazole	2.0×10^{-5}	−5.6
Ofloxacin	1.0×10^{-7}	+4.8
Enoxacin	1.0×10^{-7}	+8.0
Ciprofloxacin	1.0×10^{-7}	>10
Norfloxacin	1.0×10^{-7}	>10

3.4. Method validation

Under the optimal conditions, the calibration graphs for the determination of ENRO were constructed. The fluorescence intensity of the system showed a good linear relationship with the concentration of ENRO in the range of 1.0×10^{-9} to 2.0×10^{-6} mol L^{-1} ($r^2 = 0.9992$), the linear regression equation was IF = 150.54c ($\times 10^{-7}$ mol L^{-1}) + 7.10 ($n = 9$), and the limit of detection (LOD, S/N = 3) was 3.0×10^{-10} mol L^{-1}. A comparison with some recently reported methods for determining ENRO in the sensitivity and linear range was summed up in Table 2. It could be seen from this table that the sensitivity and linear range of this method was better than most of the reported methods [2,4,6,9,10,13–16,23].

The limit of quantification (LOQ) for ENRO in milk was determined by using a spiked milk at a concentration of 8.4×10^{-8} mol L^{-1}, detailed process was as follows: extracted with the sample preparation process, detected with synchronous fluorescence spectrometry, and evaluated by the criterion that the signal to noise ratio (S/N) should be more than 10 for quantification purposes. The LOQ was estimated to be 2.8×10^{-8} mol L^{-1} (10 μg L^{-1}) for ENRO. The sensitivity determined in milk sample seems not very high, this is because there are some residual proteins and other organic substances still existing in the prepared sample solution even if the deproteinization reagent have been employed in the sample preparation process, which will cause an increase in the fluorescence background, and result in the reduction of sensitivity.

The accuracy and precision of the method was examined by determining ENRO in the spiked milk samples at different concentrations. The mean recoveries were in the range of 82.4–90.3% with RSD less than 9%.

Within-day accuracy was evaluated continuously by performing five replicates within a day for determining recoveries in the same spiked milk sample at different concentrations. As shown in Table 3, within-day RSD of recoveries was between 4.7% and 8.7%.

Table 2
Comparison of sensitivity and linear range between the proposed method and some reported methods for the determination of ENRO.

Methods	Linear range (mol L^{-1})	LOD (S/N = 3) (mol L^{-1})	References
Spectrophotometry	5.6×10^{-6} to 3.3×10^{-5}	2.8×10^{-6}	[2]
FI–CL[a]	2.2×10^{-8} to 1.0×10^{-5}	8.3×10^{-9}	[4]
HPLC–fluorescence detector	8.3×10^{-8} to 4.2×10^{-5}	2.6×10^{-8}	[6]
AdCdSWV[b] (electroanalysis)	1.0×10^{-8} to 8.0×10^{-8}	3.3×10^{-10}	[9]
QDs-cFLISA[c]	2.3×10^{-8} to 2.8×10^{-7}	7.0×10^{-9}	[10]
ENRO-chloranil (CT reaction)	1.4×10^{-7} to 2.8×10^{-6}	4.7×10^{-8}	[13]
Solid-phase spectrofluorimetry	5.6×10^{-10} to 1.1×10^{-8}	1.1×10^{-10}	[14]
ENRO–Tb^{3+}–SLS[d]	8.9×10^{-9} to 2.1×10^{-7}	2.5×10^{-9}	[15]
ENRO–Eu^{3+}–Phen[e]–SDBS[f]	–	2.3×10^{-7}	[16]
Immunosensor	2.8×10^{-9} to 2.8×10^{-6}	2.8×10^{-9}	[23]
This method	1.0×10^{-9} to 2.0×10^{-6}	3.0×10^{-10}	

[a] Flow injection–chemiluminescence.
[b] Adsorptive cathodic stripping square wave voltammetry.
[c] Competitive fluorescence-linked immunosorbent assay (cFLISA) based on quantum dots (QDs).
[d] Sodium lauryl sulfate.
[e] 1,10-Phenanthroline.
[f] Sodium dodecylbenzene sulfonate.

Table 3
The precision and accuracy of the proposed method for determining ENRO in the spiked milks.

Sample	Added ($\mu g\,L^{-1}$)	Found[a] ($\mu g\,L^{-1}$)	Recovery[a] (%)	R.S.D. (%)	
				Within-day[a]	Day-to-day[b]
Milk[c]	50	41.2 ± 3.5	82.4	8.7	9.0
	100	84.7 ± 5.6	84.7	6.6	7.0
	200	180.6 ± 8.4	90.3	4.7	4.9

[a] The mean of five replicates in a day ($n = 5$).
[b] $n = 3$ replicates × 5 days.
[c] 10 times diluted and deproteined with acetonitrile.

Table 4
Determination of total residual amounts of ENRO and its metabolic product (CPFX) in different fresh milks purchased from local supermarket.

Samples	Found[a] ($\mu g\,L^{-1}$)	R.S.D (%, $n = 3$)
Fresh milk 1	126.0 ± 10.1	8.0
Fresh milk 2	61.3 ± 5.3	8.6
Fresh milk 3	138.9 ± 20.0	14.4
Fresh milk 4	69.9 ± 6.0	8.6
Fresh milk 5	235.0 ± 28.5	12.1

[a] The mean of three replicates.

Day-to-day accuracy was also evaluated by performing the determination of recoveries in the same spiked milk sample at different concentrations each day on five consecutive days. Day-to-day RSD of recoveries was between 4.9% and 9.0%.

3.5. Sample determination

The five fresh milk samples produced by different companies were purchased from a local supermarket. These fresh milk samples were dealt according to the above sample preparation procedure, and determined by the proposed method. Table 4 showed all the ENRO residues in these milk samples were within 61.3–235 $\mu g\,L^{-1}$ with RSD less than 15%. The results indicated that only 40% of the milk samples its ENRO residues (total amount of ENRO and its metabolic product, CPFX) was less than MRLs (100 $\mu g\,L^{-1}$), and one milk sample its ENRO residue was even more than 2 times of MRLs.

The ENRO injection from Xinchang Gufeng Veterinary Co. Ltd., China, was directly determined by the proposed method after diluted properly. Meanwhile, the same sample was respectively determined by the method of Tb^{3+} luminescent probe [24]. The results were shown in Table 5. Table 5 showed that the concentration of the sample determined by the presented method was in accordance with that of the Tb^{3+} luminescent probe and its mark concentration determined by other method. So, the selectivity and precision of this method were satisfactory.

3.6. Luminescence mechanism

Fig. 2 showed that when Y^{3+} ions were added to the ENRO solution, the fluorescence intensity of the ENRO–Y^{3+} system was significantly enhanced, which indicated that a coordination compound between ENRO and Y^{3+} ions was formed. Moreover, the fluorescence spectra of the system had a blue-shift about 3 nm. Also, it could be seen from Fig. 5 that when Y^{3+} solution was added to the ENRO solution, the absorption of ENRO at 274 nm was obviously increased, which indicated that the formation of the ENRO–Y^{3+} complex could increase the absorption and resulted in the fluorescence enhancement of the system. Therefore, it was reasonable to assume that the fluorescence enhancement was due to the formation of complex, the fluorescence of the system belonged to Y^{3+}-perturbed luminescence of ligand or L*–L luminescence. It might be also supposed that Y^{3+} as a rare-earth ion with strong binding ability was bound to carbonyl and carboxyl groups of ENRO and closed a six-membered ring [25,26], which could explain the cause of the absorption increase when Y^{3+} was added to the ENRO system.

Table 5
Determination of enrofloxacin in the injection sample by different methods.

Drug sample	The proposed method			Tb^{3+} luminescent probe			Mark conc. (%)
	Found (%)	Mean (%)	RSD (%)	Found (%)	Mean (%)	RSD (%)	
Enrofloxacin injection	2.42, 2.40, 2.45, 2.45, 2.44	2.43	0.9	2.50, 2.48, 2.63, 2.52, 2.55	2.54	2.4	2.5

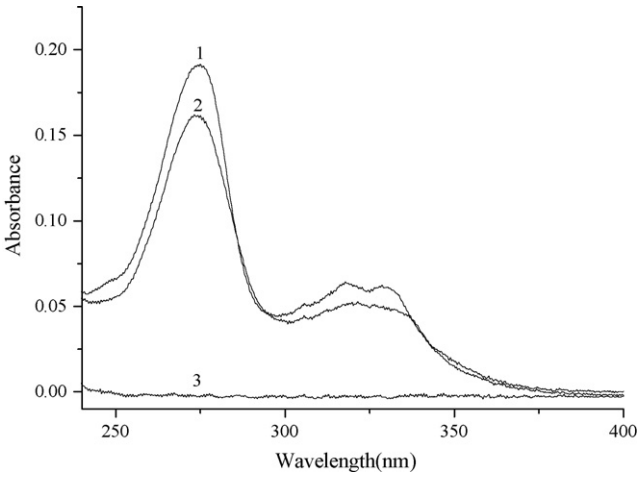

Fig. 5. Absorption spectra. (1) ENRO–Y^{3+}, (2) ENRO, (3) Y^{3+}. Conditions: Y^{3+}: 1.0×10^{-4} mol L^{-1}, ENRO: 5.0×10^{-6} mol L^{-1}, HMTA: 0.1 mol L^{-1}, pH = 6.3.

4. Conclusions

Based on this new yttrium (III)-perturbed luminescence system, a simple, rapid and sensitive synchronous fluorescence method is put forward for the determination of enrofloxacin (ENRO) in the pharmaceutical formulation and its residue in milk. This method can determine the total amount of ENRO and CPFX in a single measurement, so it can be used as a rapid screening for judging whether the ENRO residues in milks exceed MRLs or not. In addition, when this method is applied to determine ENRO in the pharmaceutical formulation, its selectivity is also very good. Common metal ions, rare-earth ions and some pharmaceuticals, which are usually used together with ENRO, do not interfere with the determination of ENRO under the actual conditions.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20877067) and Zhejiang Provincial Natural Science Foundation of China (No. R507212).

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