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# Determination of trace fluoroquinolones in water solutions and in medicinal preparations by conventional and synchronous fluorescence spectrometry

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**Abstract:** Simple, rapid and sensitive and synchronous fluorescence spectrometry (SFS) were developed for determination the fluoroquinolones of ciprofloxacin (CIP), norfloxacin (NOR) and enrofloxacin (ENR) separately in water solutions and in medicinal preparations. The optimized wavelength intervals between the emission and excitation wavelengths were 170 nm, 160 nm and 170 nm for CIP, NOR and ENR, respectively. The different experimental parameters affecting the synchronous fluorescence intensities of the three fluoroquinolones were carefully studied. Under the optimal conditions, good linearity was obtained over the range of 0.01 to 1.20 mg/L, 0.005 to 0.45 mg/L and 0.005 to 0.60 mg/L for the CIP, NOR and ENR, and with good relative standard deviations below 1.9% (n=9). In addition, the detection limits for CIP, NOR and ENR were 0.17 μg/L, 0.013 μg/L and 0.055 μg/L, respectively. What is more, compared with the conventional fluorescence spectrometry, the SFS could detect lower concentrations of each fluoroquinolone. Moreover, the proposed SFS were validated and successfully applied for the quantitative assay of each fluoroquinolone in medicinal preparations.

**Keywords:** Fluoroquinolone; Synchronous Fluorescence Spectrometry; Ciprofloxacin; Norfloxacin; Enrofloxacin.

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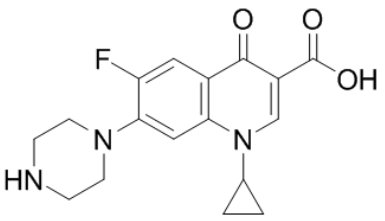
## 1 Introduction

Fluoroquinolones (FQs), such as ciprofloxacin (CIP) norfloxacin (NOR) and enrofloxacin (ENR) are a group of synthetic antibacterial agents with broad-spectrum activity for both of gram positive and negative bacteria through inhibition of DNA gyrase [1-3]. Thus, they are commonly selected as antibacterial medicines to treat many diseases [4,5]. However, they can act as toxic substances that cause direct toxicity or allergic reactions in hypersensitive individuals. In addition, micro-organism resistance to these drugs can occur [6-8]. The widespread occurrence of FQs as contaminants in the aquatic environment has garnered increased attention in recent years. Consequently, attention has been paid to their determination in various environmental waters, e.g., waste water, surface water, ground water and drinking water [9-12].

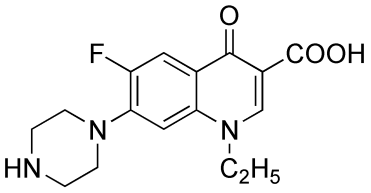
Several methods such as spectrophotometry [13], UV [14,15], CE with ECL detection [16,17], voltammetry [18], HPLC [19-21], HPLC-FLD [22], and NMR have been reported to determine the FQs [23]. Still, most of the above methods are time-consuming and quite tedious which need large volumes of organic solvents with sophisticated instrumentation. Among these methods, the fluorescence spectrometry assay is comparatively simple, time-saving, and especially suitable for the detection with many analytes in a trace amount [24-28]. fluorescence spectrometry has a growing importance in medicine analysis. The main advantage of fluorescence spectrometry is its high sensitivity and selectivity as compared to other spectroscopic methods [29]. Synchronous fluorescence spectrometry was first introduced by Lloyd [30]. And the synchronous fluorescence spectra, acquired by scanning excitation and emission monochromators simultaneously, can be modeled by Gaussian distributions of intensity versus energy [31]. Synchronous fluorescence spectrometry (SFS) has several advantages over conventional

fluorescence spectrometry, including simple spectra and low interference. In addition, the SFS is a simple modification of the conventional fluorescence method that affords a higher selectivity by narrowing the spectral bands and simplifying the spectra [31]. Synchronous fluorescence spectra are measured using both excitation and emission monochromators locked together at a fixed wavelength interval (Δλ, $\Delta\lambda=\lambda_{em}-\lambda_{ex}$) and simultaneous scanning. In this method, the selectivity is increased, and a high sensitivity is maintained [32,33].

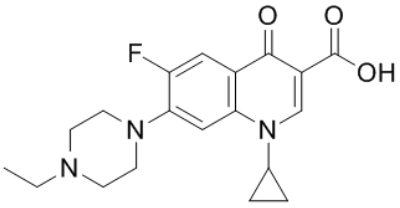
To the best of our knowledge, few studies have focused on the determination of FQs using SFS. The current study aimed to establish new SFS to determine typical FQs in water solutions by selecting CIP, NOR, and ENR as the model compounds (Figure 1). In addition, under the optimal experimental conditions, the CIP, NOR and ENR contents in medicinal preparations dissolved in water solutions were separately determined using the new SFS.




A: CIP




B: NOR




C: ENR

**Figure 1: Chemical structures of CIP, NOR and ENR**
(A) CIP (B) NOR (C) ENR.

# 2 Experimental

## 2.1 Reagents and chemicals

The chemicals of CIP, NOR and ENR were obtained from Sigma-Aldrich. Stock solutions of each FQ were prepared by dissolving the solutes in ultrapure water and stored in a refrigerator. The working solutions of the individual CIP, NOR and ENR were prepared by appropriate dilution of the stock solutions of each FQ in ultrapure water before use. Other chemicals used were of analytical reagent grade.
In this experiment, two kinds of each FQ in the medicinal preparations were investigated. There were two CIP tablets called A (Hangzhou Minsheng Pharmaceutical Co., Ltd.) and B (Zhejiang Jingxin Pharmaceutical Co., Ltd.), two NOR tablets called C (Hangzhou Minsheng Pharmaceutical Co., Ltd.) and D (Jiangxi Tianzhihai Pharmaceutical Co., Ltd.) and two ENR injections called E (Sichuan Jixing Animal Pharmaceutical Co., Ltd.) and F (Shanxi Ruicheng Animal Pharmaceutical Co., Ltd.), respectively.

## 2.2 Apparatus

All the fluorescence measurements were recorded with a luminescence spectrometer (Hitachi F-4600, Japan), equipped with a xenon arc lamp, grating excitation and emission monochromators and a Hitachi F-4600 recorder. Fluorescence measurements were performed using the standard 1×1 $cm^2$ quartz cells. The excitation and emission slits of each FQ were set to 5 nm. The scan speed was 1200 nm $min^{-1}$, and the PMT voltage was 700 V.

## 2.3 Selection of the optimal Δλ for each FQ by the SFS

The working solutions of each FQ were added into the fluorescence spectrophotometer. In addition, the synchronous fluorescence spectra of each FQ was collected by simultaneously scanning the excitation and emission monochromators in the range of 200 to 500 nm at room temperature. The optimal value of each FQ in this study was investigated by changing the Δλ in the range of 10 to 190 nm in an interval of 10 nm. Finally, the optimal Δλ for each FQ was selected based on which wavelength showed the strongest fluorescence intensity and the best synchronous fluorescence spectrum. The synchronous fluorescence measurements were performed in triplicate for each sample.

### 2.4 Analysis of each FQ by the SFS in the medicinal preparations

In this study, the experimental methods for processing the tablets of CIP and NOR comprised the following steps: (1) An accurately weighed amount of A, B, C and D were separately transferred into a clean dry 100 ml flask and about 80 ml ultrapure water was added. (2) The flasks for each of the tablet were centrifuged for approximately 10 min at a speed of 4000 rpm. (3) The supernatants of the individual A and B were determined by the established SFS and the established conventional spectrometry for CIP, respectively. And the supernatants of the individual C and D were determined by the established SFS and the established conventional spectrometry for NOR, respectively. All the experiments were repeated three times at room temperature.

In this work, the processing methods for the ENR injections were as follows. A certain volume of E and F were separately diluted with ultrapure water into a clean dry 100 ml volumetric. Then, the solutions of the individual E and F were determined by the established SFS and the established conventional spectrometry for ENR, respectively. All the experiments were repeated three times at room temperature.

Ethical approval: The conducted research is not related to either human or animal use.

## 3 Results and Discussion

### 3.1 Fluorescence excitation and emission spectra of CIP, NOR and ENR in water solutions

In this work, the conventional fluorescence excitation and emission spectra of CIP, NOR and ENR in water solutions were obtained, respectively. The experimental results indicated that the fluorescence excitation and emission spectra of CIP, NOR and ENR were basically identical, because of their similar molecular structures (Figure 1). It was found that each FQ showed two different excitation peaks and only one emission peak. In addition, the maximum excitation wavelengths of CIP, NOR and ENR were 277 nm, 276 nm and 277 nm, and the emission wavelengths of CIP, NOR and ENR were 445 nm, 444 nm and 444 nm, respectively.

For this study, the working solutions of CIP, NOR and ENR were diluted by their stock solutions, respectively. In addition, the CIP was selected as the example, and

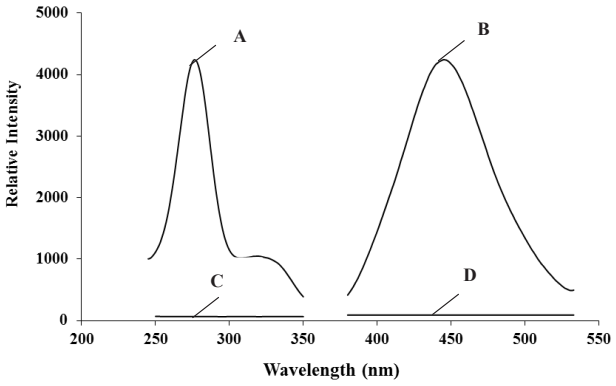



**Figure 2:** The conventional fluorescence excitation (A) and emission (B) spectra of CIP in water solutions. (A) Fluorescence excitation spectrum of CIP in water solutions; (B) Fluorescence emission spectrum of CIP in water solutions; (C) Fluorescence excitation spectrum of solvent; (D) Fluorescence emission spectrum of solvent.

the conventional fluorescence excitation and emission spectra of CIP in water solutions were shown in Figure 2. As can be seen in Figure 2, the autofluorescence of the solvent (C and D) was very weak and did not affect the results of CIP determination. Moreover, the same experimental results were also obtained that the autofluorescence of the solvent was very weak and did not affect the determinations of NOR and ENR, respectively. Thus, a series of the standard solutions of each FQ, in which the concentration of one component was variable and the other was fixed, were prepared and determined under the instrumental parameters. As shown in Table 1, a good linear relationship was obtained for each FQ, between the fluorescence intensity and the concentration of the analyte. Finally, new conventional fluorescence spectrometry for the separate determination of CIP, NOR and ENR in water solutions were established, respectively.

### 3.2 Synchronous fluorescence spectra of CIP, NOR and ENR in water solutions

It is well known that the selection of Δλ is an important parameter to obtain a good sensitivity and selectivity [29-31]. In SFS both the excitation and emission monochromators are scanned simultaneously in such a manner that a constant wavelength interval (Δλ) is kept between emission and excitation wavelengths [32]. In this study, it was investigated by changing Δλ in the range from 0 to 190 nm in an interval of 10 nm (A: 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm; B: 70 nm, 80 nm, 90 nm, 100 nm, 110 nm, 120 nm; C: 130 nm, 140 nm, 150 nm, 160 nm, 170 nm, 180 nm, 190 nm). The experimental

**Table 1: Performance data for the determination of CIP, NOR and ENR concentrations in water solutions.**

| Method | Linear Range (mg/L) | Calibration Equation | Correlation Coefficient | Detection Limit (μg/L) |
|---|---|---|---|---|
| 1 | 0.01~1.20 | $y^a = 8047x^b + 162.49$ | 0.9989 | 0.17 |
| 2 | 0.10~1.0 | y = 8198.6x – 323.89 | 0.9995 | 0.25 |
| 3 | 0.005~0.45 | y = 19696x + 199.07 | 0.9979 | 0.013 |
| 4 | 0.025~0.35 | y = 27732x + 51.378 | 0.9997 | 0.022 |
| 5 | 0.005~0.60 | y = 18243x + 173.08 | 0.9987 | 0.055 |
| 6 | 0.05~0.80 | y = 18153x + 49.048 | 0.9992 | 0.062 |

1: Synchronous spectrometric method for determination of CIP in water solutions.
2: Conventional spectrometric method for determination of CIP in water solutions.
3: Synchronous spectrometric method for determination of NOR in water solutions.
4: Conventional spectrometric method for determination of NOR in water solutions.
5: Synchronous spectrometric method for determination of ENR in water solutions.
6: Conventional spectrometric method for determination of ENR in water solutions.
[a] Relative fluorescence intensity of each FQ
[b] Concentration of each FQ (mg/L)

results indicated that the optimized Δλ for CIP, NOR and ENR were also basically identical in water solutions. They were 170 nm for CIP, 160 nm for NOR and 170 nm for ENR, respectively. In addition, each FQ had only one synchronous fluorescence peak in their optimized Δλ. They were 277 nm for CIP, 276 nm for NOR and 275 nm for ENR, respectively. In this experiment, the CIP was selected as the example to show its synchronous fluorescence spectrum with the different Δλ (Figure 3). In addition, experimental results also showed that the synchronous fluorescence intensity of the solvent was notably weak in the optimized Δλ and did not interfere with the spectra of CIP, NOR and ENR in water solutions. Moreover, the synchronous fluorescence intensities of each FQ in the optimized Δλ were also basically identical to their conventional fluorescence intensities. Furthermore, the width of the half-wide spectral band for the synchronous fluorescence spectra became much narrower than that of the conventional emission spectra for each FQ. Figure 4 showed the synchronous fluorescence spectrum and the emission spectrum of CIP in water solutions. Thus, the experimental results demonstrated the potential to use SFS to determine the concentrations of CIP, NOR and ENR in water solutions, respectively.

In the study above, the optimized Δλ for each FQ was observed. Then, further studies were investigated the synchronous fluorescence properties of different concentrations of each FQ for their optimal Δλ. Under identical experimental conditions, the synchronous fluorescence intensities varied with the various concentrations of each FQ. In addition, within a certain range, the fluorescence intensities linearly increased when the exposure quantities of CIP, NOR and ENR increased. Thus, the new SFS to directly determine CIP, NOR and ENR in water solutions were successfully established, respectively. Moreover, the calibration equations, linear ranges, correlation coefficients, and detection limits of the established methods for each FQ were shown in Table 1. Compared with the conventional fluorescence spectrometry for the determination of each FQ in water solutions, the SFS had lower limits of detection. Furthermore, the linear ranges of the SFS for determination of each FQ were much larger than that of the conventional fluorescence spectrometry (Table1).

## 3.3 Using SFS for direct determination the contents of CIP, NOR and ENR in medical preparations

In this research, the established SFS and the established conventional spectrometry were tried to directly determine the contents of CIP, NOR and ENR in medical preparations. In order to contrast, two types of CIP tablets (A and B), NOR tablets (C and D) and ENR injections (E and F) were able to opt for further studies. After being pretreated with different ways, the water solutions of A, B, C, D, E and F were obtained, firstly. Secondly, the synchronous fluorescence spectra of the water solutions for each FQ were determined in the optimal Δλ. The experimental results demonstrated that the optimal Δλ was 170 for A and B, 160 for C and D, and 170 for E and F. In addition, they were generally identical to the standard solutions of CIP, NOR and ENR, respectively. Further

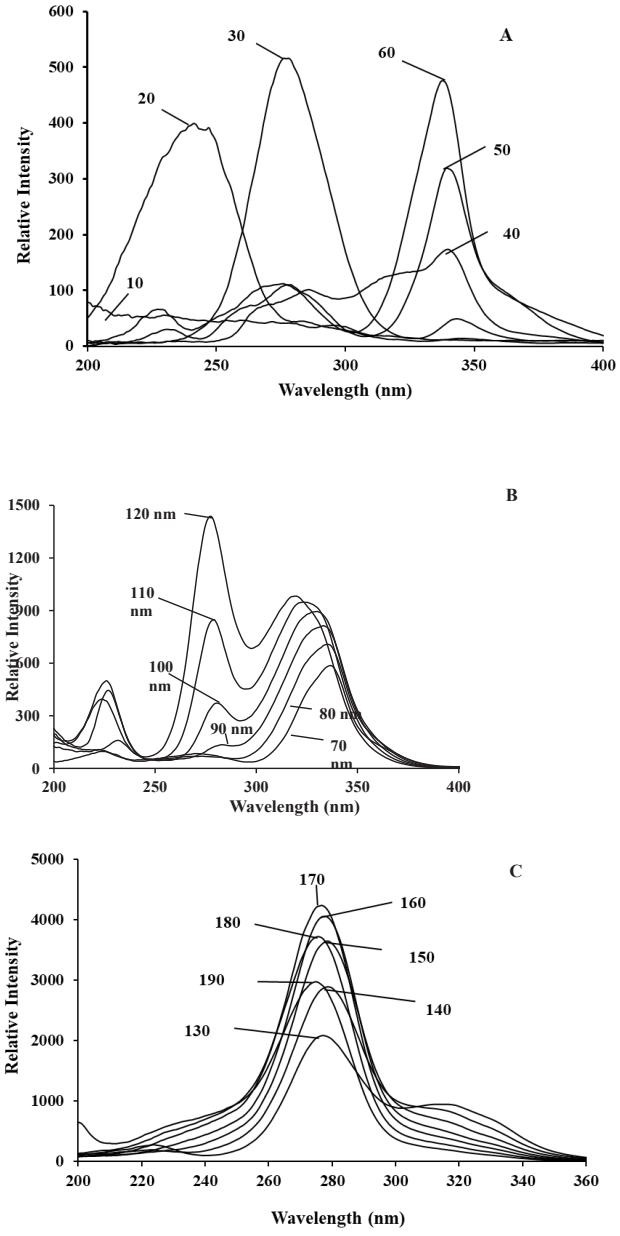



**Figure 3:** Synchronous fluorescence spectra of CIP in water with different Δλ. (A) Δλ=(10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm); (B) Δλ=(70 nm, 80 nm, 90 nm, 100 nm, 110 nm, 120 nm); (C) Δλ=(130 nm, 140 nm, 150 nm, 160 nm, 170 nm, 180 nm, 190 nm).

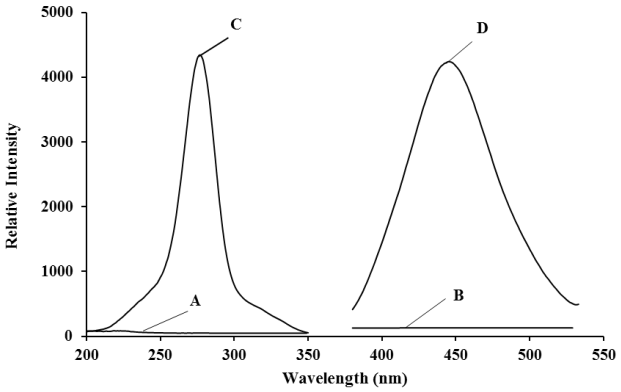



**Figure 4:** The synchronous fluorescence spectrum and the emission spectrum of the conventional fluorescence for CIP in water solutions. (A) Synchronous fluorescence spectrum of solvent; (B) Emission spectrum of solvent; (C) Synchronous fluorescence spectrum of CIP in water solutions; (D) Emission spectrum of CIP in water solutions.

results also indicated that the other ingredients mixed in each medical preparation did not produce synchronous fluorescence signals in the optimal Δλ. The CIP was also selected as the example in this study. And the synchronous fluorescence spectra of the standard solution of CIP, A and B in the optimal Δλ were shown in Figure 5.

As can be seen from the Figure 5, the synchronous fluorescence intensities of the other ingredients mixed in A and B were notably weak with no distractions of the synchronous fluorescence spectra of standard solution of CIP, A and B in water solutions (Figure 5). Finally, the contents of A, B, C, D, E and F were separately determined using the newly established SFS. The following were the results of the experiment: (1) The quantities of CIP in one tablet of A and B were 0.27 g and 0.28 g, respectively, and the values were basically identical to the contents from the medical instructions (0.25 g and 0.25 g). (2) The quantities of NOR in one tablet of C and D were 0.09 g and 0.12 g, respectively, which agreed with the contents from the medical instructions (0.10 g and 0.10 g). (3) The quantities of ENR in one bottle of injection of E and F were 0.10 g/ml and 0.11 g/ml, respectively, which were very consistent with the medical instructions (0.10 g/ml and 0.10 g/ml). Therefore, the proposed SFS successfully extended to applying into calculation the concentrations of CIP, NOR and ENR in medical preparations, respectively. In addition, the established conventional spectrometric methods were also used to determine the quantities of CIP, NOR and ENR in their medical preparations, respectively. And the results also indicated that the values of each FQ determined by the conventional spectrometric methods were basically identical to the contents from their medical instructions.

## 4 Conclusions

The SFS is a technique used to analyse dissolved fluorescent substances in water samples with satisfactory recoveries. This method is rapid, sensitive, and inexpensive and does

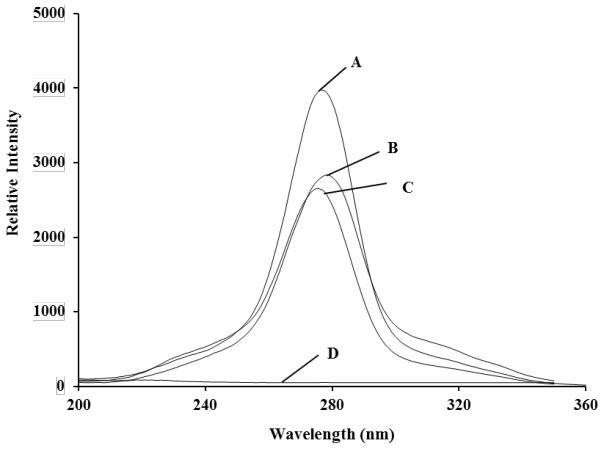



**Figure 5:** The synchronous fluorescence spectra of the standard CIP (277 nm) and two types of CIP tablets (A: 278 nm; B: 276 nm) mixed in water solution. (A) Synchronous fluorescence spectrum of the standard CIP; (B) Synchronous fluorescence spectrum of the CIP tablet of A; (C) Synchronous fluorescence spectrum of the CIP tablet of B; (D) Synchronous fluorescence spectrum of the other ingredients in water solution.

not necessitate complicated sample pretreatment with large amounts of organic solvents. In this study, The SFS were utilized for direct determination the content of each FQ in water solutions. Experimental results also indicated that the SFS could determine lower concentrations of CIP, NOR and ENR in water solutions compared with the conventional fluorescence spectrometry. Finally, the newly established SFS methods were successfully applied to determine the contents of CIP, NOR and ENR in medical preparations. In conclusion, the SFS might have great potential for development as *in situ* techniques to determine FQs in the aquatic environmental in which the other ingredients do not produce synchronous fluorescence signals. They also might be convenient methods to investigate the fluorescence behaviors of FQs in the aquatic environmental and simultaneous determination of two or more FQ components in the near future.

**Conflicts of Interest:** We declared that we have no conflicts of interest to this work.

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