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Simultaneous determination of norfloxacin and lomefloxacin in milk by first derivative synchronous fluorescence spectrometry using Al (III) as an enhancer

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ABSTRACT

A novel method for the simultaneous determination of norfloxacin (NFLX) and lomefloxacin (LFLX) in milk samples was developed by using first derivative synchronous fluorimetry. The synchronous fluorescence ($\Delta\lambda = 160$ nm) spectra and first derivative synchronous fluorescence spectra of NFLX, LFLX and their mixture were studied. The zero-crossing method was utilized to measure the first derivative value of the derivative spectrum. The zero-crossing points were located at 275.0 nm for NFLX and at 283.8 nm for LFLX, in first derivative synchronous fluorescence spectra. Therefore, 283.8 nm and 275.0 nm were selected for the determination of NFLX and LFLX. The first derivative values varied linearly with the concentrations in the range of 1.68×10^{-8}–5.64×10^{-6} mol L^{-1} for NFLX and 1.89×10^{-8}–6.19×10^{-6} mol L^{-1} for LFLX. The detection limits were 5.03×10^{-9} mol L^{-1} for NFLX and 7.58×10^{-9} mol L^{-1} for LFLX. The proposed method is reliable, selective and sensitive, and has been used successfully in the simultaneous determination of NFLX and LFLX in milk samples, whose results were in good agreement with those obtained by HPLC.

1. Introduction

Norfloxacin and Lomefloxacin are two fluoroquinolones (FQs) antibiotic drugs widely used in the treatment of respiratory diseases and enteric bacterial infections in humans and in food-producing animals such as cattle, because of their features of eury-antibacterial spectrum, hadro-antibacterial activity, high blood concentrations and eu-absorption by oral application [1,2]. There has been a progressive increase in the use of FQs in animal production, which has inevitably caused residues in animal origin foodstuff. The residual drugs would cause vivo-accumulation if human has used these foodstuffs long-term [3–5]. These drugs and their metabolites are chemical substances with different degrees of toxicity, and present potential. Consequently, they have potential carcinogenesis, teratogenesis, mutagenesis and drug tolerance, which may impact severely the health and development of human [6]. People already pay close attention to the issue of FQs residues [3]. Under Council Regulation EC 2377/90 (as amended) the European Union (EU) has established maximum residue limits (MRLs) ranging from 30 to 1900 $\mu g\,kg^{-1}$ for several species, including poultry, porcine and tissue types. Residue levels of FQs in foods of animal origin are routinely monitored in the EU under the member states National Residue Control Plan (NRCP). Therefore, it is significant to develop new high-sensitivity methods for the determination of FQs.

Various methods have been utilized for this determination. These methods include electrochemical analysis [7,8], fluorimetry [9–12], immunization [13], micellar liquid chromatography [14], HPLC [15–18], HPLC-MS [19,20], chemiluminescence-chemometrics [21], etc. Though these methods are valuable and each method has its own advantages, there are still some disadvantages in these methods. For example, Kowalski et al. [22] had reported a HPLC method for the determination of NFLX in tissues (muscle, liver, and fat) of chickens. The detection limit was 2.5 ng mL^{-1} of homogenate. Whereas, it was expensive and the large amount of organic solvents being used were toxic. Furthermore, Tong and Xiang [23] had reported the fluorescence intensity of the Tb^{3+}-SDBS (sodium dodecyl benzene sulfonate) was greatly enhanced by NFLX, and a sensitive fluorimetric method for determining the NFLX was established. The linear range of NFLX was 5.0×10^{-9}–2.0×10^{-6} mol L^{-1} with a detection limit of 1.2×10^{-9} mol L^{-1}. But there was a disadvantage of the poor stability of the probe. Moreno-González [24] reported a new method for the determination of NFLX by flow injection analysis based on photoinduced chemiluminescence detection. This method was sensitive, but many factors, such as the weight ratios of mixture and the flow rate of reagent solution, influenced the determination results. The simultaneous determination of NFLX and LFLX has rarely been reported using synchronous fluorimetry.

Synchronous fluorescence spectrometry (SFS) is a powerful tool for simultaneous multicomponent determinations without

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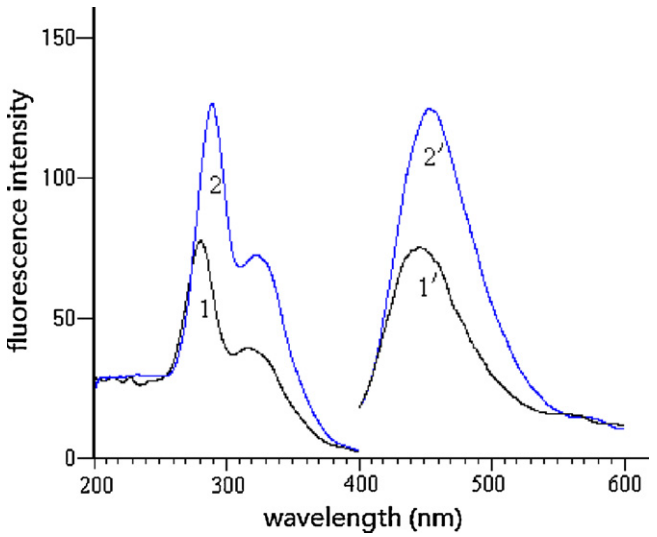

Fig. 1. The excitation (1 and 2) and emission (1′ and 2′) fluorescence spectra. 1, 1′: NFLX (6.26×10^{-7} mol L^{-1}): λ_{em} = 446.0 nm, λ_{ex} = 280.3 nm; 2, 2′: LFLX (1.29×10^{-6} mol L^{-1}): λ_{em} = 450.0 nm, λ_{ex} = 288.3 nm.

samples pre-treatment [25–28]. Although SFS is sensitive, selective, and has better resolving power in the determination of two-component, they fail in analyzing very complex mixtures where there is spectral loss as a result of the interference of the different species in the sample. Both the NFLX and the LFLX solutions have intrinsic fluorescence. Simultaneous determination of NFLX and LFLX in diverse matrixes by traditional fluorescence spectrometry or synchronous fluorescence spectrometry are hampered due to spectral overlap of their coordination (Figs. 1 and 2).

The derivative fluorescence spectrometry relies in the differentiating of normal spectrum by mathematical transformation of fluorescence spectral curve into a derivative (first- or higher derivatives) [29]. The combination of synchronous and derivative techniques reveals high sensitivity and selectivity because of narrow spectral bandwidth in relation to emission spectrum and amplitude of the secondary spectrum signal which is easily masked by predominant spectrum. This combination technique usually provides more defined fingerprints than traditional spectrofluorimetries, permits discrimination against broadband interferents, and eliminates the influence of background or matrix [30]. It has

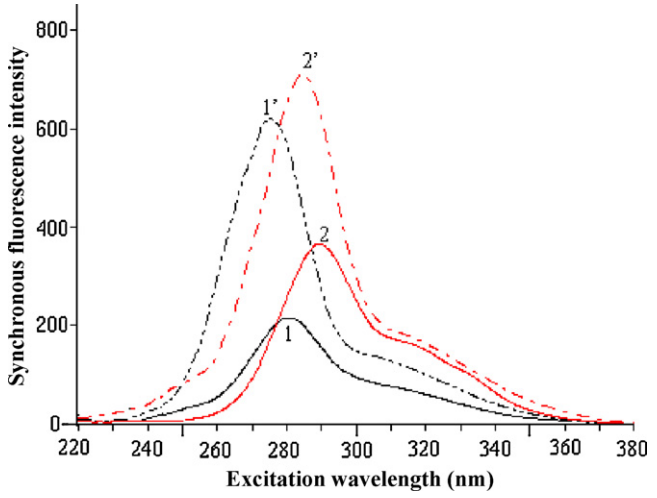

Fig. 2. The synchronous fluorescence spectra ($\Delta\lambda$ = 160 nm) of NFLX and LFLX. 1, 1′: NFLX (3.13×10^{-6} mol L^{-1}); 2, 2′: LFLX (6.18×10^{-6} mol L^{-1}); 1, 2: in the absence of Al^{3+}; 1′, 2′: in the presence of Al^{3+} of 4.8×10^{-4} mol L^{-1}.

been extensively applied to the simultaneous determination of mixtures with overlapping spectra [31,32] and is playing an important role in the multi-component analysis of mixtures.

In this paper, we actualized the simultaneous determination of NFLX and LFLX in milk samples by the first derivative synchronous fluorescence spectrometry. According to our experimental results, since the fluorescence intensity of NFLX and LFLX are very weak, so it is very necessary to select a fluorescent enhancer for the determination of them. The Al (III) was applied to the co-luminescence system [33] for determination of ciprofloxacin. In this research, the experimental results show that the fluorescence intensity of NFLX or LFLX was enhanced obviously when Al (III) was added to their solutions. Therefore, Al (III) was selected as an enhancer to enhance the fluorescence intensity of NFLX or LFLX and improve peak shape of the fluorescence spectrum. Based on the enhancing effect of Al (III), a sensitive, accurate, simple and rapid determinations method was proposed for the simultaneous determination of NFLX and LFLX in their mixtures. The proposed method has additional advantages of less time-consuming, simple instrument, high sensitivity and good selectivity resulting from eliminating low-frequency background and blank signal of samples. The method has succeeded in the analysis of NFLX and LFLX in milk samples, and the fluorescence enhance mechanism was initially discussed.

2. Experimental

2.1. Apparatus

All the fluorescence measurements were performed on a Hitachi 4500 spectrofluorimeter (Japan) equipped with a 10 mm quartz cell. Both excitation and emission bandpass of 10.0 nm, scan speed of 2400 nm s^{-1} and a PMT voltage of 400 V were set.

HPLC [34] (Dionex P680, USA) equipped with UV detector was used for accuracy assessment. The chromatographic separation was performed with a Diamonsil^{TM} C_{18} column (150 mm × 4.6 mm, 5 μm, particle size) by 10 μL sample injections and with a mobile phase of 20:80 (v/v) of acetonitrile and phosphate buffer at a flow rate of 1.0 mL min^{-1}, the column oven temperature was set at 30 °C and detection wavelength of the UV detector was set at 280 nm.

PB-20 standard pH meter (Sartorius Scientific Instruments (Beijing) Co., Ltd.), AB204-S electronic analytical Balance (Mettler-Toledo Instruments (Shanghai) Co., Ltd.) were used in this experiment.

2.2. Chemicals

The stock calibration solutions of NFLX and LFLX were prepared by dissolving an appropriate amount of NFLX and LFLX (Beijing Chemical Reagent Company, China) in HCl, and then diluting to 100 mL with doubly distilled water. The working calibration solutions of 1.25×10^{-4} mol L^{-1} for NFLX and 2.58×10^{-4} mol L^{-1} for LFLX were prepared by diluting the stock solution with doubly distilled water.

The Al^{3+} solution (8.0×10^{-3} mol L^{-1}) was prepared by dissolving 3.001 g of $Al(NO_3)_3$ (Guangzhou Chemical Reagent Company, China) in 1000 mL of 4×10^{-3} mol L^{-1} HNO_3. pH 4.0 of HAc–NaAc buffer solution was prepared by mixing 82 mL of 0.2 mol L^{-1} HAc solution and 18 mL of 0.2 mol L^{-1} NaAc solutions.

All experiments were performed with analytical reagent grade chemicals and doubly distilled water.

2.3. Pre-treatment of milk samples

Milk samples were pretreated using an appropriate modification of the liquid extractions method [35]. A 30 mL aliquot of acidified methyl cyanide (MeCN) of pH 2.0 was added to 6.0 mL of