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Determination of quinolones residues in prawn using high-performance liquid chromatography with $Ce(IV)–Ru(bpy)_3^{2+}–HNO_3$ chemiluminescence detection

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Abstract

A novel method was developed for the determination of quinolone (QN) residues such as ofloxacin, norfloxacin, ciprofloxacin and lomefloxacin by high-performance liquid chromatography (HPLC) coupled with chemiluminescence (CL) detection. The procedure was based on the chemiluminescent enhancement by QNs of the $Ce(SO_4)_2–Ru(bpy)_3^{2+}–HNO_3$ system. The separation was carried out with an isocratic elution using the mobile phase of 3:15:82 (v/v/v) acetonitrile–methanol–ammonium acetate buffer (containing 7.5×10^{-4} M TBAB, 0.8% (v/v) TEA and 1.0×10^{-4} M ammonium acetate, pH 3.65) at a flow rate of 1.0 ml/min. For the four QNs, the detection limits at a signal-to-noise of 3 ranged from 0.36 to 2.4 ng/ml. The relative standard deviations for the determination of QNs ranged from 1.6 to 4.5% within a day ($n = 11$) and from 3.7 to 6.2% in three days ($n = 15$), respectively. The method was successfully applied to the determination of QNs in prawn samples. The possible mechanism of the CL reaction was also discussed briefly.
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1. Introduction

The quinolone antibiotics (Fig. 1) are synthetic antimicrobial agents with a broad spectrum of activity widely used in human and veterinary medicine. Extensive use of antibiotics in veterinarian medicine and medicated feed play a crucial role in intensive animal production in food-producing animals and farmed fish such as prawn, salmon and catfish [1,2]. It leads to a significant increase in antibiotic resistance and allergic reactions, having therefore important consequences on public health. Moreover, quinolone-induced acute arthropathy has been observed in several animal species. Although severe cases of arthropathy have been observed only rarely in humans, incidents of transient arthralgia have also been reported [3]. These observations have precluded the use of quinolones in children and pregnant women. To safeguard human health, the European Union set a Maximum Residue Limits (MRLs) of 30 ppb for the sum of enrofloxacin and its metabolite, ciprofloxacin, in muscle, kidney and liver [4]. Because of the great varieties of quinolones, and the possibility of trace residues in edible tissue, it is necessary to develop sensitive multi-residue screening methods for the determination of quinolones (QNs).

The methods for the determination of QNs in biological samples have been reviewed [5–7]. Numerous biological and chemical techniques such as microbiological assay [8], spectrophotometry [9], fluorimetry [10–12], electrochemical detection [13], capillary electrophoresis (CE) [14–16], high-performance liquid chromatography (HPLC) [17–25] and flow-injection–chemiluminescence methods [26–28] have been reported. The analysis of QNs has traditionally been performed using microbiological methods. However, these techniques are slow and suffer from poor precision and specificity. Now most of analytical methods developed for multi-residue quinolones detection is focused on liquid chromatography with different detection modes such as spectrophotometry [17,18], fluorescence [19–23] and mass spectrometry (MS) [24,25] because they

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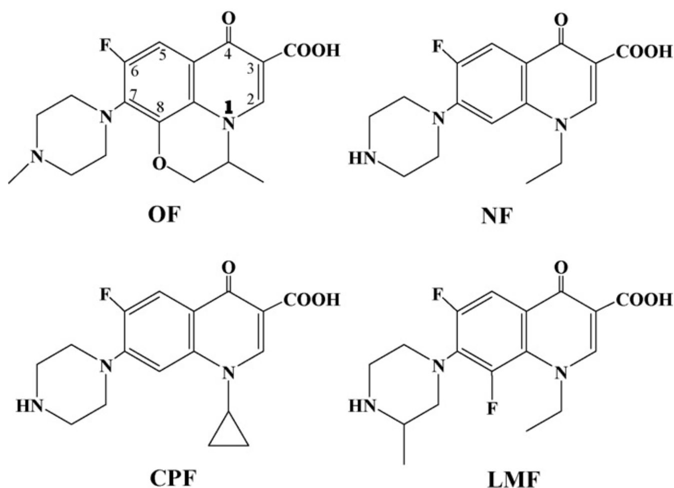

Fig. 1. Structural formulae of QNs.

can offer the advantages of better sensitivity and specificity. Of these detection modes, mass spectrometry can offer high sensitivity and selectivity for the determination of QN residues, but the instrumentation is expensive.

In recent years, chemiluminescence (CL) has become an attractive detection method for liquid chromatography due to its high sensitivity and wide linear working ranges, which can be obtained with relatively simple instrumentation. Francis and Adcock [29] reviewed the CL methods for the determination of ofloxacin involving in acidic $Ru(bpy)_3^{2+}$ system, alkaline $KMnO_4$ system and $NaNO_3$–H_2O_2 system, etc. However, these methods are restricted to flow-injection procedure and cannot be used for simultaneous determination of several QNs. Moreover, sensitivity is not good enough for the determination of QN residues. In the present work, we found that QNs could strongly enhance the CL of the $Ce(SO_4)_2$–$Ru(bpy)_3^{2+}$–HNO_3 system, the enhanced CL signal was much stronger than the $Ce(SO_4)_2$–$Ru(bpy)_3^{2+}$ system reported by Aly et al. [27] using flow-injection CL method, and the detection limits were lower than that of reported. To our knowledge, there is no report using HPLC–CL to detect QN residues. On this basis, a highly sensitive method was developed for the simultaneous determination of QN residues in prawn by coupling HPLC with this CL reaction.

2. Experimental

2.1. Chemicals and solutions

Methanol and acetonitrile were of HPLC grade and provided by Jiangsu Hanbang Technology Company (Jiangsu, China). $Ru(bpy)_3Cl_2\cdot 6H_2O$ was purchased from Sigma (Sigma, USA). Ofloxacin (OF), norfloxacin (NF), ciprofloxacin (CPF) and lomefloxacin (LMF) were obtained from the Institute of Pharmaceutical and Biomaterial Authentication of China (Beijing, China). Tetrabutyl ammonium bromide (TBAB), triethylamine (TEA), $Ce(SO_4)_2$ and HNO_3 were analytical-reagent grade and obtained from Shanghai Chemicals Company (Shanghai, China).

Stock solutions of OF, CPF and LMF (0.1 mg/ml) were prepared weekly using double distilled water, while stock solution of NF (0.1 mg/ml) was prepared weekly using methanol–double distilled water for its poor solubility. The stock solutions were stored at 4 °C in a refrigerator. A stock solution of $Ru(bpy)_3^{2+}$ (1.0×10^{-2} M) was prepared using double distilled water. The solution of $Ce(SO_4)_2$ (1.0×10^{-3} M) was prepared by dissolving $Ce(SO_4)_2$ in 3.6×10^{-2} M sulfuric acid. All working solutions were freshly prepared each day with double distilled water. The HPLC mobile phases were freshly prepared each day, filtered through a 0.22-μm membrane filter (Xinya Company, Shanghai), and then degassed before use.

2.2. Instrumentation

The schematic diagram as described previously [30] illustrated the HPLC–CL detection system used in our experiments. The HPLC system was Agilent 1100 series (Agilent Technologies, USA), including a binary pump, a thermostatic column compartment, a diode array detector (DAD), a manual sample valve injector with a 100-μl loop, and an analytical column (Zorbax Eclipse XDB-C_8, 150 mm × 4.6 mm i.d., 5 μm, Agilent Technologies, USA). CL detection was conducted on a flow-injection–chemiluminescence system (Remax, China) consisting of a model IFFM-D peristaltic pump, a mixing tee and a model IFFS-A CL detector equipped with a glass coil (i.d. 1 mm, 13 cm length used as reaction coil and detection cell), and a photomultiplier. The data from the CL detector were acquired by Agilent Interface 35900E and processed by Chemstation A.08.03 running on a DELL smartpc 100 personal computer.

Absorption spectra were acquired using a Shimadzu UV-2401 Spectrophotometer (Tokyo, Japan). CL spectra were measured on a Shimadzu RF-5301 Spectrofluorometer (Tokyo, Japan). pH measurement was carried out on a REX pHS-3B meter (Shanghai REX, China).

2.3. Procedure

The quinolones were separated by a XDB-C_8 column at 30 °C with an isocratic elution at a flow rate of 1.0 ml/min. The mobile phase consisted of 3:15:82 (v/v/v) acetonitrile (A)–methanol (B)–ammonium acetate buffer (C containing 7.5×10^{-4} M TBAB, 0.8% (v/v) TEA and 1.0×10^{-4} M ammonium acetate, pH 3.65). The separation was carried out in 8 min and typical retention time of OF, CPF, NF and LMF was 5.6, 6.2, 6.8 and 7.6 min, respectively. The UV–vis detector was set at 278 nm for NF and CPF and 295 nm for OF and LMF. The column effluent from DAD was first mixed with the solution of HNO_3 at a mixing tee via a PEEK tube (600 mm × 0.25 mm i.d., Agilent Technologies), then mixed with the combined stream of $Ru(bpy)_3^{2+}$ and $Ce(SO_4)_2$ solution. The CL solutions of HNO_3, $Ce(SO_4)_2$ and $Ru(bpy)_3^{2+}$ were pumped at a flow rate of 5.0 ml/min, respectively. Light emission was monitored by the photomultiplier tube. The quantitative determination was based on the net CL intensity $I = I_S - I_0$, where I_S is the CL intensity in the presence of QNs and I_0 is the CL intensity of blank signal.

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