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Study on Fluorescence Property of Sparfloxacin Derivatizing System and its Application

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Abstract: In acid medium, sparfloxacin is oxidized by nitrous acid, then reacts with hydrobromic acid to form a new fluorescence substance, which can emit the strong fluorescence, which is 151 fold more than that of sparfloxacin itself. By this, a new sensitive method for the determination of sparfloxacin in human urine by derivative-synchronous fluorescence is presented with good results.

Keywords: Sparfloxacin, nitrous acid, derivative-synchronous fluorescence, human urine.

Sparfloxacin (SPFX) is the fourth generation of the antibacterial quinolones. Due to its intensive and efficient antibacterial activity compared to the common quinolones [1], it has been widely used in clinical practice. Nevertheless, because of the structural differences between SPFX and other quinolones, the fluorescence of SPFX is so weak that it can not be determined by means of fluorescence spectra. Hence, it will be of great interest to make a detailed study on its fluorescence enhancement whether in clinical practice or in pharmacokinetics. As to the analytic methods in determining quinolones by spectrophotometry [2-4], there have been reported a lot , such as HPLC [5-6], and thin-layer chromatography (TLC)[7] *et al*. but using fluorescence spectra as a means to determine trace amount of SPFX in human body has not been developed. In this work ,we made a detailed investigation on SPFX-HNO_2-HBr system .It has been found that SPFX can be oxidized promptly by nitrous acid, then reacts with HBr further to yield a new fluorescence substance, which can emit strong fluorescence. Based on this, a new sensitive method for the determination of SPFX by derivative-synchronous fluorescence was established. Meanwhile, the structure of this new fluorescence substance was characterized and the mechanism of reaction was discussed. Experiments show that the method presented in this work not only is high-sensitive but easy to operate and good in selection is very convenient to carry out.

Experimental

The fluorescence spectra were obtained on a PE LS-50B spectofluorometer. Excitation and emission of bandpass 5 nm were employed and a 1 cm cuvette was employed for measurements. The acidity of the system was measured on a pHs-2 meter. Sparfloxacin from the Institute of Pharmaceutical Products Control of China was prepared as 5×10^{-4}

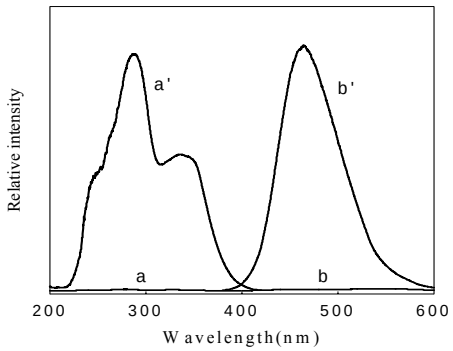
mol/L stock solution. $NaNO_2$ was prepared as 5×10^{-3} mol/L stock solution which was stored in a brown reagent bottle and kept in refrigerator. 0.2 mol/L of HBr and CuBr water solutions were freshly prepared, 0.2 mol/L of H_2SO_4 were also prepared as stock solution. All the reagents used in this work were of analytical grade. H_2O was doubly distilled in a sub-boiling distiller.

An aliquot of SPFX and 0.5 mL H_2SO_4 stock solutions were in turn piped into a comparison tube of 25 mL, into which 0. 25 mL $NaNO_2$ solution was added dropwise in an ice-bath at the temperature range of 0℃～5℃ then 0.2 mL HBr and 0.2 mL CuBr were added and shaken to mix well the solution. The mixture solution was kept for 20 min within the above temperature range, then heated for 1 h over a hot water bath and diluted to final volume with pH 3.1 NaAc-HAc buffer solution. After setting for 10 min, fluorescence of the solution was measured. The excitation and emission wavelengths were fixed at 287 nm and 465 nm, respectively.

Result and Discussion

As shown in **Figure 1**, SPFX can emit only very weak fluorescence at 538 nm. While after oxidation by nitrous acid and reaction with HBr, the fluorescence intensity of solution was enhanced in 151 fold higher than that of SPFX itself. Meanwhile, the wavelength of fluorescence emission is blue-shifted to 465 nm, which indicates that a new fluorescence substance is formed. With quinine sulfate (0.05 mol/L of H_2SO_4, $_f$ =0.55) as standard solution, the fluorescence quantum production rate of SPFX and this new fluorescence were determined as 0.0072 and 0.1692, respectively.

Figure 1. Excitation and emission of SPFX and SPFX–HNO_2–HBr:



a ，b : SPFX $\lambda_{ex}/\lambda_{em}$=284 nm/538 nm, a′，b′ : SPFX–HNO_2–HBr $\lambda_{ex}/\lambda_{em}$=287 nm/465 nm, $[H_2SO_4]=4\times10^{-3}$ mol/L, $[NaNO_2]=5\times10^{-5}$ mol/L, [HBr] $=1.6\times10^{-4}$ mol/L,
a ,b ,a′ ,b′ [SPFX] $= 5\times10^{-5}$ mol/L in pH 3.1 NaAc –HAc buffer.

The experiments show that when 0.15～0.3 mL of 5×10^{-3} mol/L $NaNO_2$, 0.4～0.6 mL of 0.2 mol/L H_2SO_4, 0.1～0.5 mL of 0.2 mol/L HBr are used, the fluorescence intensity of the system is highest and stable. So in this experiment, The choice of 0.5 mL H_2SO_4, 0.25 mL $NaNO_2$ and 0.2 mL HBr was adopted.

The time of SPFX-HNO_2-HBr kept in the ice-bath will directly affect the fluorescence behavior of product and it is similar to that for heating. It was shown by

experiments that the fluorescence intensity reached its maximum and the fluorescence quantum production rate was highest when the ice-cooling time and heating time for 20 min and 1 hour, respectively.

When fixed the concentration of SPFX as 5×10^{-5} mol/L using different buffer solution to control the pH of system, the fluorescence intensity reached its maximum at pH value of 3.0～3.3. With the reduction of pH, fluorescence intensity was decreased From the above results determination of SPFX in human urine by derivative-synchronous fluorescence is presented. Figure 2 gives the synchronous spectra of mixture of SPFX with blank urine fixed as =70 nm. It can be seen that the resolution is still not very well due to the serious overlap of fluorescence emission between internal hormone and new fluorescence substance. However, the best resolution is obtained by means of the first-order derivative fluorescence spectra for all detected components(see **Figure 3**).

Figure 2. Synchronous fluorescence synchronous spectrum

Figure 3. Ist order derivative fluorescence spectrum

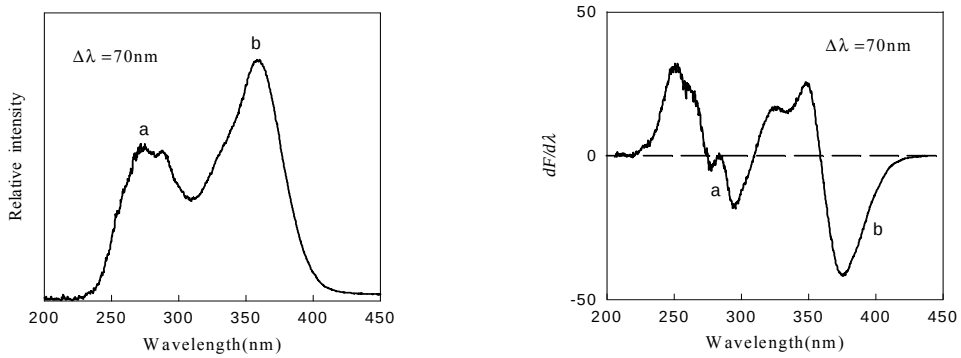


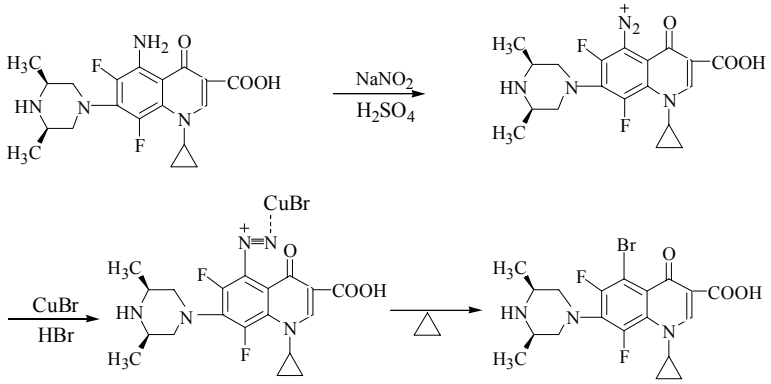
Figure 2 , 3: a. Internal hormones in human urine; b. SPFX–HNO_2–HBr $[H_2SO_4]=4\times10^{-3}$ moL/L ; $[NaNO_2]=5\times10^{-5}$ moL/L**;** [HBr] $=1.6\times10^{-4}$ moL/L ; pH=3.1 ; $C_{SPFX}=1.0$ g/mL.

According to the experimental procedure early, the mixed standard , SPFX and blank urine were scanned by fixing the excitation wavelength within the range of 200～450 nm, then measured using peak–zero method with 375 nm (–)as determining wavelengths. The results shows that plot of $dF/d\lambda$ *versus* [SPFX] possess a good linearity in the concentration range of 0. 1μg/mL～4. 0 μg/mL, the regression equation was $dF/d\lambda = -7.328\ C_{spfx}-0.275$, with a correlation coefficient of 0.9997 and detection limit 0.1μg/mL. 2.5 mL urine sample of healthy person was exactly piped and determined SPFX for five times in parallel. The results show that RSD is 1.4 % and recovery 97.3 %.

At last, the reaction mechanism was explored in the following. According to references [8], SPFX is oxidized by HNO_2 , then reacts with HBr to prepare a new pure fluorescence substance. According to the results measured by IMPACT–410 (NICOLET)

In the IR spectrum, (KBr)the peaks at ν_{N-H}3450 cm^{-1}, 3360 cm^{-1}, ν_{C-N} 1290 cm^{-1},

ν_{N-H} 1575 cm^{-1} all disappear. This indicates that -NH_2 group of the molecular has been substituted by atom –Br. It can be demonstrated from the strong absorption peak at ν_{C-Br} 1106 cm^{-1}. So the fluorescence enhancement mechanism of SPFX derivatizing reaction can be proposed as follows.



The structures of SPFX and other quinolones were compared. It is found that the 5-NH_2 is the ultimate reason for SPFX fluorescence disappearing. In SPFX molecules, 5-NH_2 is easily protonized in acid media to form $–NH_3^+$, herefor, it can be translated from a strong electron donating groups to a strong electron-attacting groups. On the other hand, in SPFX molecules, 5-NH_2 and 4-OH easily form intramolecular hydrogen bond, which reduces the degree of freedom of plane π-bond. Finally it leads to the great reduction of fluorescence yield. From our investigation, as the 5-NH_2 is substituted by –Br, the electron cloud of benzoate is not further reduced and the intramolecular hydrogen bond is also disappeared. Therefore, it can cause the freedom of plane π-bond and to enhance the fluorescence of SPFX molecules.

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