Chinese Chemical Letters Vol. 12, No. 11, pp 1007 – 1010, 2001
http://www.imm.ac.cn/journal/ccl.html

Determination of Sparfloxacin in Human Urine by Reversed-Phase High Performance Liquid Chromatography With Nitrous Acid and Hydroiodic Pre-Column Derivatization

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Abstract: Sparfloxacin can be oxidized by nitrous acid, then react with hydroiodic acid to form a fluorescent derivative. Based on this, a reversed-phase high performance liquid chromatographic pre-column derivatization new method is described for the determination of sparfloxacin in human urine. The linear range is 0.05 mg/L to 4.0 mg/L, the recoveries are 91.5%~95.7% and the RSD is 1.2%~4.2%. The results showed that this method is suitable for the determination of sparfloxacin in human urine.

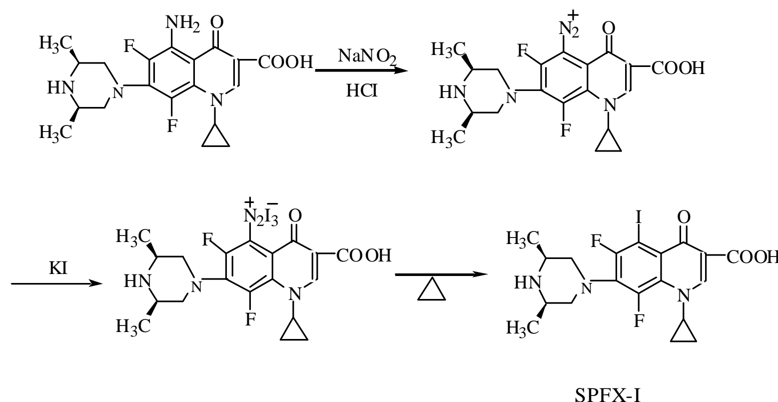
Keywords: Sparfloxacin, HPLC, pre-column derivatization, human urine.

Quinolone drugs, due to the aromatic systems with benzoheterocycle in their molecules, can usually emit strong fluorescence. However, contrary to this, sparfloxacin (SPFX) as the fourth generation quinolone products, can only emit weak fluorescence, although it possesses a similar structure to quinolones. At present, the majority of SPFX determinations have been carried out using techniques such as colorimetry[1] and spectrophotometry[2-3]. For the high performance liquid chromatography (HPLC), it has been reported only confined to the UV detector[4-6], while a pre-column derivatization of high performance liquid chromatography with fluorescence detection has not been used so far for direct SPFX determination.

In this paper, SPFX was oxidized by nitrous acid, then reacted with potassium iodide to form a fluorescence sparfloxacin derivative (SPFX-I), which can emit a strong fluorescence, the sparfloxacin derivative reaction can be proposed as follows:

Experimental

All chromatographic measurements were completed on an American HP-1050 high performance liquid chromatograph equipped with HP-1106 fluorescence detection. The excitation and emission wavelength selected was 275 nm and 464 nm, respectively. The excitation and emission slits both were 5 mm. The amount of sample-injection was 15 μL.



SPFX solution and 0.5 mL of 0.2 mol/L HCl were in turn pipetted into a 25 mL flask, then settled in an ice-water bath. 0.25 mL of 5×10^{-3} mol/L $NaNO_2$ and 0.2 mL of 0.2 mol/L KI solutions were then slowly added and the mixture was shaken to obtain a homogeneous solution. After setting for 10 min at 0~5°C, the flask was put in a sub-boiling water bath for 1 h again, then cooled down to room temperature. At this time, 2.5 mL methanol solution was pipetted into the flask and the mixed solution was diluted to volume with the mobile phase. Under the chromatographic conditions selected, 15 μL sample was injected for chromatographic measurements.

Results and discussion

Optimization of experimental conditions

The experiments showed that when 0.15~0.3 mL of 5×10^{-3} mol/L $NaNO_2$, 0.25~0.8 mL of 0.2 mol/L HCl, 0.1~0.4 mL of 0.2 mol/L KI were used, the fluorescence intensity of the system was highest and most stable. So in this experiment, the choice of 0.5 mL HCl, 0.5 mL $NaNO_2$ and 0.3 mL KI was adopted. The time of SPFX—$NaNO_2$—KI kept in the ice-bath as well as the heating time would directly affect the fluorescence intensity of the reaction system. It was shown by experiments that the fluorescence intensity reached its maximum and the apparent fluorescence quantum yield was the highest when the ice-bathing time and heating time were 10 min and 1 hours, respectively.

In this experiment, several conventional reversed-phase HPLC such as CH_3OH-H_2O and CH_3CH_2CN-H_2O, were used in order of priority for SPFX separation. However, to our regret, SPFX can not be separated from internal hormones in human urine if CH_3CH_2CN-H_2O being used as the mobile phase, even if the proportion of mobile phase is adjusted or adding the appropriated acid or salt. While if CH_3OH-H_2O being used as mobile phase, SPFX could be separated from internal hormones in human urine but chromatographic peak tailing severely. And the peak tails more sever with the decrease in the volume of fraction methanol. In the meantime, the retention time is lasted, and up until the volume fraction of methanol reaches 55%, only can make the

chromatographic peak of SPFX derivative systematic and separated from other components.

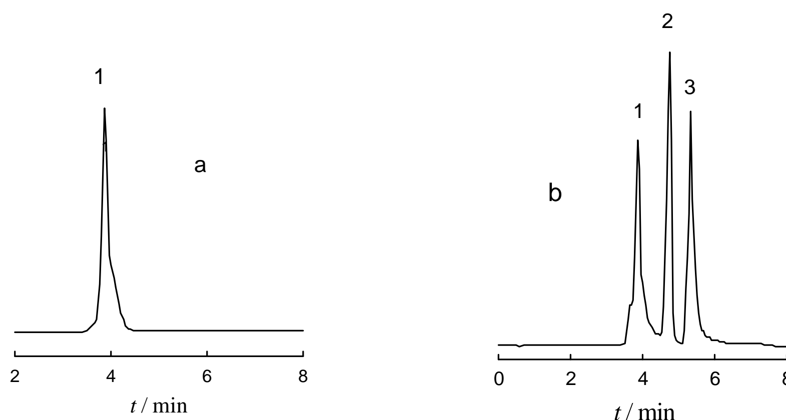
Experiments show that the optimum proportion of mobile phase was 0.5 mol/L tetraethyl ammonium bromide: 0.02 mol/L phosphate: methanol (8:40:52,v/v) composition. Under this condition, SPFX derivative can be separated from internal hormones and internal standard in human urine completely. The detailed results are shown in **Figure 1.**

The response signal of SPFX was very weak in different pH solutions, but the response signal of SPFX derivative is greatly influenced by the pH of solution. When pH was adjusted to 3.0~3.4, the chromatographic peak area reached maximum. In this paper, pH 3.1 was chosen.

The mobile phase flow rate not only influenced the chromatograph response value but also the shape of peak. When the flow rate is below 0.8 mL/min, the shape of peak began to radio distortion, under which the retention time also is lasted. The peak area is over 1.2 mL/min, the response value also is decreased. So the flow rate of 1.0 mL/min was selected as the optimum elution condition in this experiment.

Under different temperature conditions t=25°C to 40°C with Δt=5°C at an interval, the influences of temperature of column on experimental were also investigated in detail. It was shown that the optimum separation temperature of column is 30°C. The separation degree is greatly decreased with the decrease in temperature and the retention time is lasted in the meantime.

Figure 1 Chromatograms of SPFX



a. Standard sample of SPFX; b. human urine.
1. Sparfloxacin derivative.
2. Internal hormones in human urine. 3. Internal standard, salicylic acid.

Calibration curve and detection limit

Based on the experimental procedure previously described, the linear regression equation, Y=0.269ρ+0.0186, then can be obtained, where the correlation coefficient r=0.9995 and the LOD is 0.05 mg/L.

Application

A 2.5 mL urine samples of a healthy subject who had taken orally SPFX tablets of 200 mg for 2 h (1#), 4 h (2#) and 8 h (3#), respectively, were added into a 25 mL flask, then 3 mL H_2SO_4 of 0.1 mol/L was added, the above solution was boiled for 15 min. Similarly, according to the procedure mentioned previously, all data can be obtained. The results are listed in **Table 1**.

Table 1 The precision and recovery for sparfloxacin in human urine (n=5)

Sample	Original amount (mg/L)	Amount added (mg/L)	Amount found (mg/L)	Recovery (%)	RSD (%)
1#	2.153	2.0	3.982	91.5	1.8
2#	1.329	1.0	2.286	95.7	1.2
3#	0.831	0.5	1.297	93.2	4.2

Acknowledgment

This work was supported by the Natural Science Foundation of Shanxi Province (No. 20011016).

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Received 27 March, 2001