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Spectrophotometric and HPLC determination of fleroxacin in tablets

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The paper describes and compares spectrophotometric and HPLC determination of fleroxacin in commercial tablets. The optimum conditions for spectrophotometric assay were found to be at pH < 3.5 (0.1 M HCl) at a wave length of 286 nm. HPLC analysis was carried out on a Beckman ODS 5 μm column in a pH 3 phosphoric acid solution (detector wave length 254 nm).

1. Introduction

Fleroxacin belongs to the third generation of multiple fluorinated antibacterial quinolone derivatives widely used in the treatment of urinary infections. This generation of fluoroquinolones has a broader spectrum of action and greater activity than nalidixic and oxolinic acid. The mechanism of their action has been extensively studied [1]. These agents have been shown to prevent bacterial DNA biosynthesis by inhibiting the bacterial enzyme DNA gyrase .

Several modern instrumental methods, including HPLC [2], GC MS [3], fluorimetry [4], polarography and adsorptive stripping voltammetry [5, 6], have been used for analysis of quinolone antibiotics of this type. Literature on fleroxacin determination shows that HPLC with spectrofluorimetric detection is the major analytical method for its determination in biological fluids [7–14].

Since the authors are aware of no literature data on the spectrophotometric and HPLC determination of fleroxacin in dosage forms, this paper describes and compares those two analytical methods for determination of fleroxacin in commercial tablets.

2. Investigations, results and discussion

2.1. Spectrophotometric determination

Fleroxacin, 6,8-difluoro-1-(2-fluoro-ethyl)-1,4-dihydro-7-(methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, like the other fluoroquinolones, has two potentially ionizable functional groups, namely the carboxylate and piperazine amino groups with similar acidity with pK_1 and pK_2, 5.59 and 8.08, respectively, as was previously determined [15]. According to the pK values, at pH lower than 3.5 and pH higher than 10 only cationic (H_2Q^+) and anionic (Q^-) forms are present in the solution. Between these pH values the cation, zwitter and anion forms are in equilibrium. Since the absorptivity of the H_2Q^+ is higher than that of the Q^- (Fig. 1), the optimum conditions for spectrophotometric determination of fleroxacin were found to be at pH < 3 (0.1 M HCl) and a wave length of 286 nm. No change in the absorption of fleroxacin solutions in 0.1 M HCl was found over two days.

Beer's low was checked by measuring fleroxacin concentrations ranging from 2–10 μg/ml and the corresponding regression equation (with standard error of intecept and slope) was:

$$y = (0.0036 \pm 0.0032) + (0.1027 \pm 5.78 \cdot 10^{-4})\,x$$

where y and x denote absorbance and concentration (μg/ml), respectively. The correlation coefficient was found to be 0.9999. Limit of detection and limit of determination were found to be 0.075 μg/ml and 0.252 μg/ml, respectively.

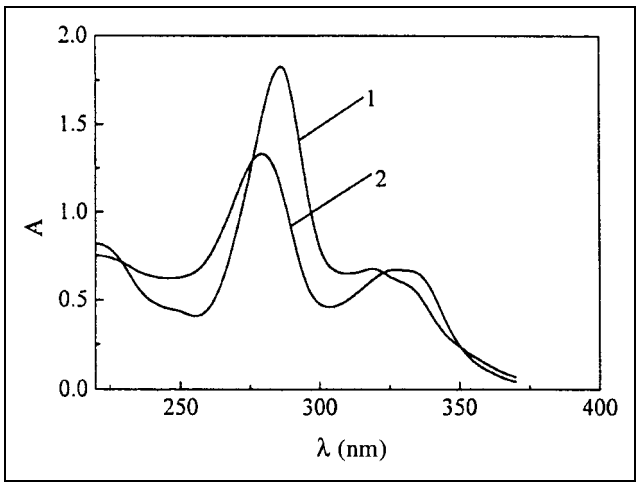

Fig. 1: Absorption spectra of pure ionic fleroxacin obtained in: 1: 0.1 M HCl (H_2Q^+); 2: 0.1 M NaOH (Q^-)

The precision of the method as determined by analyzing fleroxacin solution (5 μg/ml), expressed as a mean recovery value of 99.53% and relative standard deviation (n = 6) 0.70%, indicates very good reproducibility of the method.

The method was applied to the determination of fleroxacin in Quinodis® tablets and the results are presented in the Table.

Table: Content of fleroxacin in Quinodis® tablets[a] obtained by spectrophotometric (A) and HPLC (B) methods

	Assumed concentration[b]	Found mg per tablet	RSD% (n = 6)	Percentage of labelled content
A	3.17 μg/ml	392.3	0.72	98.08
	4.75 μg/ml	390.4	0.81	97.61
B	0.100 mg/ml	391.61	2.55[c]	97.90

[a] Label claim: fleroxacin 400 mg per tablet
[b] Fleroxacin concentration in the working solution calculated on the basis of the label claim
[c] n = 9

2.2 HPLC determination

The HPLC method was applied to the determination of fleroxacin in dosage forms. A representative chromatogram of fleroxacin obtained under the optimal conditions chosen is shown in Fig. 2. The linearity of the method was tested by measuring the peak area for five solutions

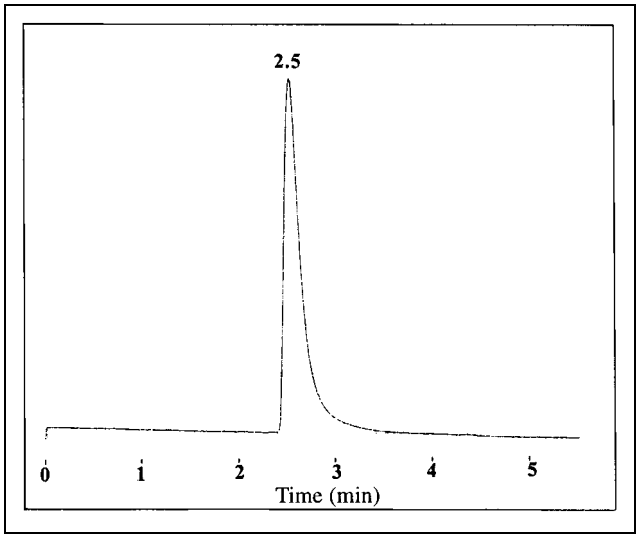

Fig. 2: Representative chromatogram of fleroxacin in Quinodis® tablets. Mobile phase: acetonitrile/water, 60 : 40; pH 3.0; flow rate 1 ml/min; UV detection at 254 nm

of fleroxacin (0.02525–0.2020 mg/ml). Results were evaluated by linear regression analysis and the corresponding equation was:

$$y = (-4216.6 \pm 5058.8) + (2.2975 \cdot 10^6 \pm 4.073 \cdot 10^4)\, x$$

where the y is the peak area and x is concentration (mg/ml), with correlation coefficient being 0.9991. Limit of detection and limit of determination were found to be 7.68 μg/ml and 25.6 μg/ml, respectively.

The precision of the method was checked by analyzing six different samples of the same fleroxacin concentration (0.1010 mg/ml). The mean recovery value was 101.2% with relative standard deviation 1.53%.

The results obtained by HPLC analysis of the same Quinodis® tablet formulation as that already analyzed by spectrophotometry are presented in the Table.

The knowledge of acid-base equilibria of fleroxacin [15] was the basic for the choice of optimal experimental conditions for both methods and permitted its spectrophotometric and HPLC determination in tablets. A good agreement was obtained by both methods applied and both methods can be used for routine determination of fleroxacin. Although HPLC is the most powerful analytical method, in this case, the simplicity and low cost of the spectrophotometric method can be its advantage.

3. Experimental

3.1. Apparatus and reagents

The following apparatus was used: a PHM-62 pH meter (Radiometer, Copenhagen, Denmark) with a combined electrode and a Beckman DU 650 Spectrophotometer.

The HPLC system consisted of Varian Star 9012 Pump and 9065 UV/vis detector. Separations were achieved on a Beckman ODS 5 μm column (250 × 4.6 mm). The mobile phase was acetonitrile/water (60 : 40, v/v). The pH of the solution was adjusted with phosphoric acid to 3.0, then filtered and degassed with He. Injection volume was 20 μl. Chromatography was carried out at a flow-rate of 1 ml/min and a detector wave length of 254 nm.

All investigations were carried out with fleroxacin produced by Hoffmann La Roche (Basel, Switzerland). Other reagents, HCl, NaOH were of analytical reagent grade from Merck. Double distilled water was used throughout.

The reagents used in the HPLC method were of HPLC quality and all other chemicals were of analytical-reagent grade (acetonitrile, phosphoric acid 85% m/m). Purified and filtered water (with a membrane filter) was used throughout.

3.2. Spectrophotometric determination of fleroxacin

3.2.1. Standard solutions

A stock fleroxacin solution (0.1 mg/ml) was prepared by dissolving fleroxacin in 0.1 M HCl. Aliquots of 0.2–1.0 ml of this solution were transferred into 10 ml volumetric flasks and diluted with 0.1 M HCl to mark. The absorbance of these solutions was measured at 286 nm against 0.1 M HCl.

3.2.2. Sample solutions

Ten Quinodis® tablets (Hoffmann La Roche, Basel) were weighed and powdered (average weight 0.5154 g); 0.1021 g of the powder was transferred quantitatively to a 200 ml volumetric flask and dissolved in 0.1 M HCl. The suspension was filtered through a filter paper (blue strip). Filtrate 2 ml was diluted with 0.1 M HCl to 100 ml. Two different aliquots (4 and 6 ml) of this solution were transferred to 10 ml volumetric flasks and diluted to the volume with 0.1 M HCl and absorbance was measured at 286 nm. The fleroxacin concentration of the working solutions were 3.17 and 4.75 μg/ml, calculated with respect to the labeled content.

3.3. HPLC determination of fleroxacin

3.3.1. Standard solutions

The stock solution containing 1.0 mg/ml of fleroxacin was prepared by weighing 10 mg of fleroxacin, 5 ml of the mobile phase was added and dissolved for 10 min in an ultrasonic bath. The final volume of 10 ml was made up by addition of mobile phase.

0.25, 0.5, 1.0, 1.5 and 2.0 ml volumes of the stock solution were transferred into 10 ml volumetric flasks, and made up the volume of 10 ml with mobile phase. The solution was filtered through a 0.45 μm Millipore filter.

3.3.2. Sample solution

A quantity of the tablet formulation containing about 100 mg of fleroxacin was weighed and transferred to a 100 ml volumetric flask. After addition of 50 ml of mobile phase the solution was treated in an ultra-sonic bath for 20 min. The total volume of 100 ml was made up with mobile phase. The solution was then centrifuged and filtered through a 0.45 μm Millipore filter. One ml of this filtrate was transferred to the 10 ml volumetric flask and diluted to volume with mobile phase. The final concentration of the solution was 0.1 mg/ml of fleroxacin.

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