ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 145–150

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Determination of fleroxacin in human serum and in dosage forms by derivative UV spectrophotometry[1]

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Received 17 September 1997; received in revised form 18 November 1997; accepted 30 November 1997

Keywords: Fleroxacin; Antibiotic; Serum; Dosage forms; Derivative spectrophotometry

1. Introduction

Fleroxacin [6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid] is a new fluoroquinolone antibiotic that exhibits strong bactericidal activity against a wide range of Gram-negative and Gram-positive bacteria [1–3]. It possesses three fluorine atoms, two of which are directly attached to quinolone nucleus, while the third one is on the side chain. In this respect fleroxacin is different from other fluoroquinolones which have a single fluorine atom [4] (Fig. 1).

The mechanism of action of fleroxacin is based primarily on the inhibition of bacterial DNA topoisomerase II (DNA gyrase). Fleroxacin inhibits supercoiling of bacterial DNA to form double stranded DNA as well as relaxation of DNA helix. It may also inhibit DNA replication, recombination, repair and transcription. As a consequence vital bacterial metabolic pathways are disturbed thus, leading to lysis of the bacteria.

Fleroxacin is rapidly and completely absorbed after ingestion of the drug. Peak plasma concentration of approximately 5 μg ml^{-1}, is reached in 1–2 h after a single dose of 400 mg. Compared with several other quinolones, noticeably, ciprofloxacin, ofloxacin, lomefloxacin and norfloxacin, fleroxacin exhibits better bioavailability and higher plasma concentrations.

Present methods for quantification of fleroxacin and their metabolites include microbiological assay [5,6], fluorometry [7,8] and HPLC with fluorescence and UV detection [9–11]. Tissue pharmacokinetics of fleroxacin have been studied by use of ^{19}F nuclear magnetic resonance spectroscopy [12] and positron emission tomography using ^{18}F-labeled fleroxacin [13]. A HPLC method has been primarily employed for quantification of fleroxacin in biological fluids and human tissues. Some of the aforementioned methods are not suitable for routine analysis in clinical laboratories. Firstly, they require long pre-treatment of the biological samples and, secondly, expensive equipment is not readily available in many control laboratories. Therefore, the aim of the present

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[1] Presented at the 7th Meeting on Recent Developments in Pharmaceutical Analysis, Island of Elba, Italy, September 16–20, 1997.

PII S0731-7085(98)00151-4

investigation was to develop a fast, sensitive, accurate and simple procedure for determination of fleroxacin in biofluids and pharmaceutical dosage forms, without prior, tedious, extraction procedures.

In continuation of our previous determination of fluoroquinolones [14–16] in the present paper, second-order derivative UV spectrophotometry was used for the direct assay of fleroxacin in serum and in pharmaceutical forms.

2. Experimental

2.1. Reagents and standard solutions

Fleroxacin standard (purity 100%, MW 369, white crystalline powder) was a product of Hoffmann La Roche (Basel, Switzerland). Solution for intravenous infusion, 'Quinodis' (400 mg of fleroxacin per 100 ml of solution) and tablets 'Quinodis' (400 mg) were products of Hoffmann La Roche. Standard solutions of NaOH (0.1 and 2 M) were prepared from Merck (Darmstadt, Germany) reagents. Water was doubly distilled.

2.2. Apparatus

Spectrophotometric measurements were performed on a GBC (Australia), UV-VIS, model Cintra 40 double-beam spectrophotometer, interfaced to an IBM PC computer. Derivative spectra were obtained with the software supplied by the manufacturer (Spectral, Personal Spectroscopy Software). Quartz cuvettes of 1 cm pathlength were used. Working settings were: slit width 0.5 nm, scan speed 100 nm min^{-1}, time response 0.1 ms and $\Delta\lambda = 1$ nm. For the disintegration of the Quinodis™ tablets, an ultrasonic bath (Bandelin Sonorex Super, Model RK 512H) was used.

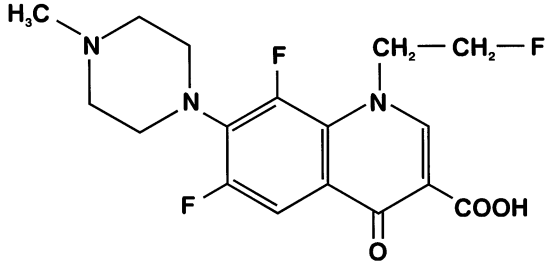

Fig. 1. Structure of fleroxacin.

2.3. Calibration graph and procedure for i.v. infusion and tablets

Stock solution of fleroxacin (1.0×10^{-3} M) was prepared by dissolving fleroxacin standard in doubly distilled water. Different volumes, 0.05–0.8 ml of the stock solution of fleroxacin were pipetted into 10 ml volumetric flasks using Eppendorf reference pipettes, diluted with 0.1 M NaOH to the mark and shaken well. The concentration range thus covered, was 1.8–30 μg ml^{-1}. The second-order derivative spectra were recorded in the 320–380 nm wavelength range against 0.1 M NaOH as a reagent blank.

The i.v. infusion solution was opened and the content was mixed in a beaker. A volume equivalent to approximately 36.9 mg of fleroxacin was pipetted into a 100 ml volumetric flask and diluted with water to the mark. Different volumes of sample solution (0.1–0.6 ml) were used in the same procedure as for calibration graph. Concentration range covered was 3.69–22.14 μg ml^{-1}.

Ten tablets were finely powdered. A weighed quantity, equivalent to 36.9 mg of fleroxacin, was transferred to a beaker, dissolved in water and sonicated for 15 min. The solution obtained was filtered into a 100 ml volumetric flask and diluted with water to give a final concentration of 1.0×10^{-3} M. Aliquots of solution (0.1–0.6 ml) were used in the same procedure as for calibration graph.

2.4. Calibration graph and procedure for serum samples

A human pool serum (1 ml) was mixed with ethanol (2 ml, 96 wt%) and with standard solution of fleroxacin, to give drug concentration of 0.5–20 μg ml^{-1}. Solubility of fleroxacin in ethanol is 10 mg 100 ml^{-1}. According to literature data [1–3] 23% of fleroxacin in serum is bound to serum proteins. Thus, addition of ethanol prevents fleroxacin binding to proteins (mainly albu-

min) and coagulate serum proteins. After deproteinization and centrifugation of samples, for 15 min at 6000 rpm speed, supernatant (1 ml) was separated and 0.055 ml of 2 M NaOH was added. The concentration range thus covered was 0.47–19 μg ml^{-1} fleroxacin. Second order UV derivative spectra of the prepared solutions were taken in the 320–380 nm wavelength range, against the serum blank, prepared as described above but without addition of the drug.

2.5. Analytical recovery

Five different concentrations of fleroxacin were added to a human serum in order to get concentrations 1.0–10.0 μg ml^{-1}. These serum samples were treated in the same way as for the calibration graph. Analytical recovery was 98.5–100.8%.

3. Results and discussion

For the determination of fleroxacin in serum and in dosage forms its strong absorption in ultraviolet was used. In Fig. 2, the zero (a) and second-order derivative spectra (b), for the three different concentrations of fleroxacin, in serum in 0.1 M NaOH are shown.

Sodium hydroxide was chosen because of enhanced solubility of fleroxacin in alkaline medium. The UV spectrum consists of two bands with the maxima at 281 nm for 1st band and 326 and 336 nm for 2nd band. Assignment of the bands in Fig. 2, was done on the basis of literature data on similar fluoroquinolones [17]. Because the absorption of serum proteins interferes with the band at 281 nm the peak at 336 nm was chosen for analysis. Since this peak is not well separated from the peak at 322–326 nm the second-order derivative spectrum was used. In this way the background absorption of the serum was also minimized.

The calibration graph for the derivative spectrophotometry was constructed by plotting peak-to-peak amplitude, in the second derivative spectrum, versus drug concentration. The amplitude was measured in 337–347 nm wavelength interval. The equation obtained through regression analysis of the data for standard solution of serum was:

$$Y = (55.7 \pm 0.1)X + (2.9 \pm 0.2)10^{-6}$$
$$(n = 7,\ r = 0.9998,\ S_x = 2 \times 10^{-6})$$

where Y is the peak-to-peak amplitude in second order derivative spectra, X is the concentration of fleroxacin in mol l^{-1} and S_x is standard error of estimation. The limit of detection, DL, defined as [18]:

$$DL = \frac{t}{b}\sqrt{S_x^2 \frac{n-2}{n-1}}$$

where n is the number of samples, b is the slope of the regression line, t is Student's value at $P = 0.05$ level of significance and S_x^2 is the variance of the fit, was found to be 30 ng ml^{-1}.

Table 1 shows the results obtained in the analysis of serum samples.

The accuracy of measurements, expressed in terms of relative error (R.E.) [19] was about 1.0% or even less, thus indicating negligible influence of serum proteins. Clinical investigations have shown that fleroxacin is rapidly and completely absorbed after oral administration. Peak plasma concentration (5.6 μg ml^{-1}) is reached approximately 1 h after administration of a single dose of 400 mg. The bioavailability is close to 100% and together with large AUC area indicate that effect of the first liver pass is negligible. Fleroxacin has long plasma half-life (10 h) and is predominantly excreted in the urine (70%). In urine fleroxacin is in an unchanged form and only 5–6% is in the form of fleroxacin *N*-oxide, *N*-demethyl fleroxacin and glucuronide [20–22]. In this way fleroxacin very slowly metabolize in plasma, allowing accurate determination even more than 10 h after administration. Thus, the proposed method for the assay of fleroxacin in serum, being simple and rapid, can be applied for the purpose of pharmacokinetic measurements without significant interference from its metabolic products.

Table 2 summarizes the results obtained in the analysis of the pharmaceutical dosage forms. For the purpose of tablet analysis, three packs from three different series of 'Quinodis' tablet were used. Two infusion ampoules form two different series of 'Quinodis' i.v. solution were used.

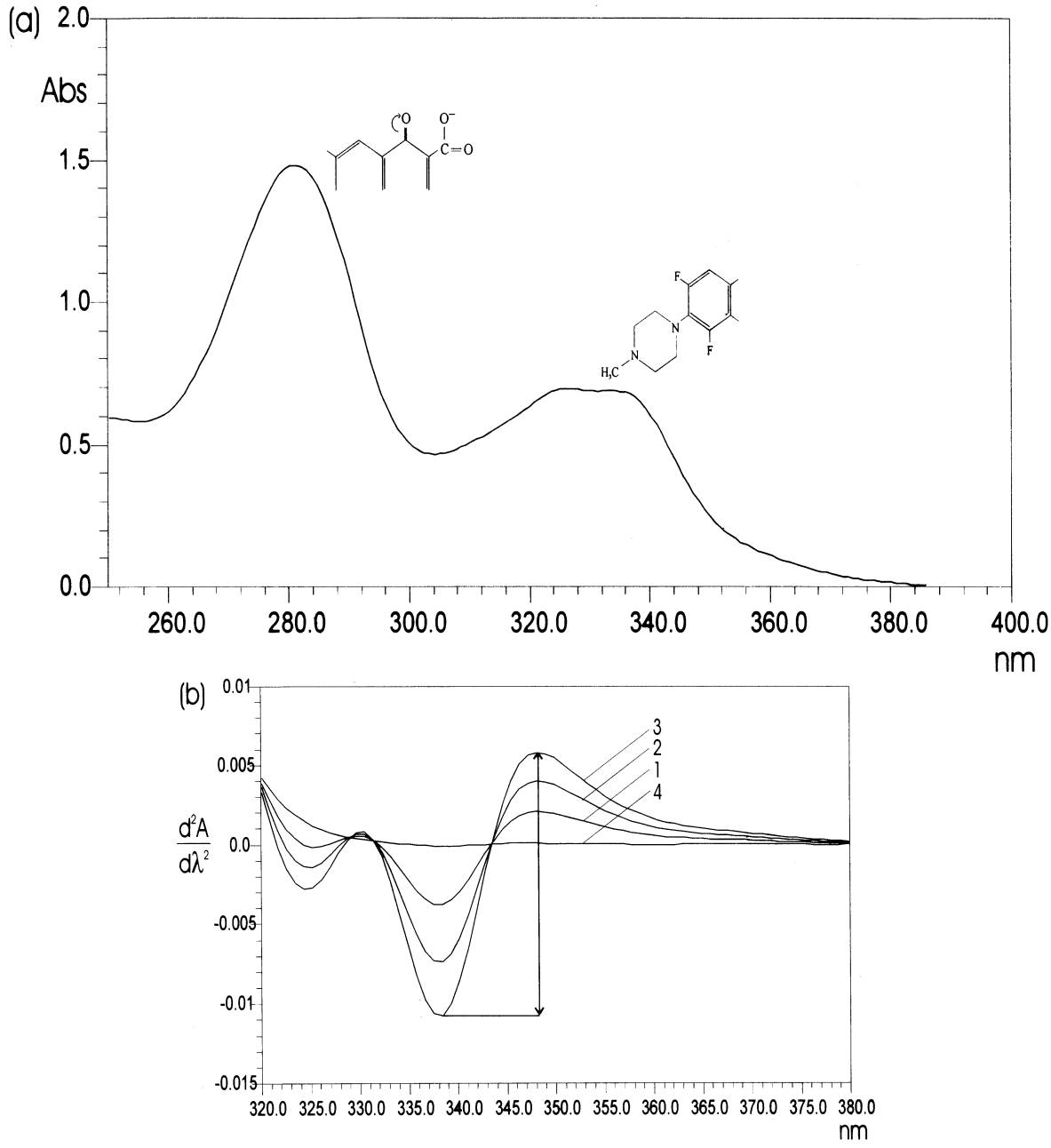

Fig. 2. Zero order (a) and second order derivative spectra (b) of fleroxacin in serum in the presence of 0.1 M NaOH and ethanol. The concentrations in derivative spectra are: spectrum 1: 12 μg ml^{-1}; spectrum 2: 23 μg ml^{-1}; spectrum 3: 35 μg ml^{-1}; spectrum 4: blank serum with no addition of fleroxacin. Concentration for zero order spectrum is 23 μg ml^{-1}.

All data represent the average of five determination. Low values of relative error and relative standard deviation of determination (R.S.D.) indicate very good reproducibility of the measurement. They also indicate that commonly used soluble excipients (starch, lactose, stearates, etc.)

Table 1
Results obtained in determination of fleroxacin in serum samples[a]

Sample	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	R.E. (%)	Recovery (%) ± R.S.D.
1	0.300	0.296 ± 0.004	1.33	98.5 ± 1.3
2	0.600	0.595 ± 0.004	0.8	99.2 ± 0.7
3	0.900	0.904 ± 0.003	0.4	100.8 ± 0.3
4	1.200	1.206 ± 0.001	0.5	100.5 ± 0.1
5	2.000	2.007 ± 0.002	0.4	100.4 ± 0.1

[a] Mean, standard deviation, relative error (R.E.) and relative standard deviation (R.S.D.) in five determinations.

Table 2
Results obtained in determination of fleroxacin in pharmaceutical forms[a]

Sample	Nominal ($mg\ ml^{-1}$)	Found ($mg\ ml^{-1}$)	R.E. (%)	Recovery (%) ± R.S.D.
Tablet 1	400	395.7 ± 1.0	1.08	99.1 ± 0.2
Tablet 2	400	400.7 ± 0.9	0.20	100.2 ± 0.2
Tablet 3	400	396.4 ± 0.7	0.90	98.9 ± 0.3
I.V. infusion (solution 1)	400	397.8 ± 0.6	0.54	99.5 ± 0.2
I.V. infusion (solution 2)	400	399.2 ± 0.4	0.22	99.8 ± 0.1

[a] Mean, standard deviation, relative error (R.E.) and relative standard deviation (R.S.D.) in five determinations.

do not interfere with fleroxacin determination. Second order derivative spectrum was chosen for dosage form analysis because the lower energy band in the spectrum of fleroxacin is broad and of complex structure (two closely situated peaks) with unfavorable intensity which makes conventional spectrophotometry unsuitable. Higher energy band in the spectrum is located too low in UV so that excipients from the formulations and even NaOH may interfere with absorption. From these reasons high energy band can not be used in analysis.

3.1. Interferences

The results presented in Table 1 refer to conditions when only fleroxacin was added to serum. As explained before their metabolites do not interfere with the determination. However, in some cases parallel therapy is required, usually with antacids or antianemic drugs. The interactions are possible between Al^{3+} containing species with fleroxacin leading to alteration of the fleroxacin spectrum. Masking with fluoride ion can be applied in order to avoid these interferences or HPLC method must be used instead. Other drugs giving strong absorption in 300–360 nm wavelength range also interfere so their simultaneous presence must be either avoided or HPLC should be used.

Acknowledgements

Financial support from the Ministry of Science and Technology of Serbia is gratefully acknowledged.

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