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Fluorimetric assay of rufloxacin in serum and in pharmaceutical formulations*

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Abstract: A simple and rapid fluorimetric method for the determination of 9-fluoro-10-[*N*-(4′-methyl)piperazinyl]-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-*d*,*e*][1–4]benzothyazin-6-carboxylic acid hydrochloride (MF 934), in serum and in pharmaceutical formulations, has been developed based on its strong fluorescence, in 0.1 N H_2SO_4, at 526 nm (excitation wavelength at 340 nm). The procedure which involves the direct dilution of the sample requires only a few minutes and the sample volume is only 20–100 μl of serum, depending on the drug concentration. Tedious sample preparation procedures such as extraction, deproteinization, or centrifugation are not necessary. The minimum concentration that can be detected is 0.3 ng ml^{-1}, the standard curve in 0.1 N H_2SO_4 was found to be linear from 0.005 to 1.5 μg ml^{-1} and from 0.01 to 0.07 g in plasma after dilution with 0.1 N H_2SO_4.

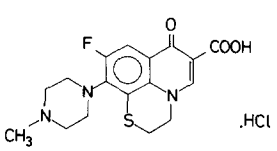
Keywords: *Spectrofluorimetric determination; rufloxacin; pharmaceutical formulations.*

Introduction

Rufloxacin (9-fluoro-10-[*N*-(4′-methyl)piperazinyl]-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-*d*,*e*][1,4]benzothyazin-6-carboxylic acid hydrochloride) (Fig. 1) is a new fluoroquinolone antibacterial agent. It is one of a large number of compounds [1] that have been synthesized and it has been selected for extended pre-clinical testing on the basis of its high clinical efficacy. Rufloxacin (R-HCl) is almost completely absorbed from the gastrointestinal tract and can be administered orally.

Typical analytical procedures for quantifying some quinolone and fluoroquinolone carboxylic acids in biological samples such as serum, urine, saliva, bile and tissues involve either extraction with solvents such as chloroform, acetonitrile [2–4], dichloromethane [5–10] or deproteinization [5, 11–14], or ultrafiltration [15, 16] generally followed by reversed-phase [2, 3, 5, 7, 9–16] or ion-exchange [6, 8] chromatography. Only one example of the direct assay of ciprofloxacin using HPLC (C-18 column) without prior extraction or clean-up procedure has been reported [17].

Figure 1



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Because rufloxacin is a highly fluorescent compound this property has been used to develop a spectrofluorimetric method for its direct analysis. The proposed method involves the direct determination of rufloxacin in 0.1 N H_2SO_4 without prior separation and is a simple and reproducible procedure that can be applied to the analysis of rufloxacin in pharmaceutical formulations and in biological samples.

Experimental

Apparatus

A Perkin–Elmer model LS-5 scanning spectrofluorimeter with a 150-W xenon lamp, interfaced with a 7700 data station, was used. The fluorescence of solutions in a 10-mm silica quartz cell was measured at the following wavelengths and bandwidth settings: 340 and 10 nm (excitation); and 526 and 10 nm (emission). All glassware was thoroughly cleaned, rinsed several times with deionized water and dried.

Reagents and samples

All reagents used were of analytical grade purity. Rufloxacin hydrochloride and 100 and 200 mg rufloxacin capsules were supplied by Mediolanum (Milan, Italy). 0.1 N Sulphuric acid and 0.1 N sodium hydroxide were prepared from Normex (Carlo Erba, Italy) reagents.

Serum samples

Serum samples from a healthy volunteer, treated with a 300 mg single oral dose of R-HCl, were taken at 0, 4, 8, 12 and 24 h after administration of the drug. A second series of serum samples, from another healthy volunteer who ingested 400 mg R-HCl on the first day and 200 mg on the second and the third days, were taken at 0, 4 and 12 h on the first day and after 4 h on the second and the third days. All these samples were kindly supplied by Mediolanum (Milan, Italy).

Calibration curves

Aqueous standards. Solutions for analysis were freshly prepared by diluting an aqueous stock solution containing 1.0 mg ml^{-1} of R-HCl to concentrations in the range 0.005–3.0 $\mu g\ ml^{-1}$ with 0.1 N H_2SO_4. The fluorescence of each solution and of 0.1 N H_2SO_4 blank were measured at 526 nm using an excitation wavelength of 340 nm and net values were plotted against concentrations.

Serum standards. A serum sample was spiked with 2.0 $\mu g\ ml^{-1}$ of drug and then diluted with 0.1 N H_2SO_4 to 0.01–0.10 $\mu g\ ml^{-1}$. Blanks values obtained by diluting the same volume of serum were subtracted from the fluorescence intensities of the diluted spiked serum. The calibration graph obtained by plotting fluorescence intensities versus concentrations was used for the determination of the concentration of drug in serum samples from healthy volunteers.

General procedure for capsules

The contents of 10 capsules were accurately weighed, well mixed and the average weight was determined. An accurately weighed amount of the powder equivalent to about 100 mg of drug was suspended in 50 ml of distilled water in a 100 ml volumetric

flask and occasionally shaken. After 30–60 min, the suspension was diluted to volume and shaken. An aliquot of this solution, filtered if necessary, was diluted with 0.1 N H_2SO_4 to a concentration in the range 0.04–0.10 μg ml^{-1}.

The concentration of the drug was read from the calibration curve prepared by using the aqueous standards.

General procedure for serum samples

Serum samples were stored at −20°C and used without any further treatment. Aliquots of 20–100 μl of the serum (depending on R-HCl concentration) were added directly to the cuvettes containing 3.4 ml of 0.1 N H_2SO_4 mixed and the fluorescence intensity measured. After subtraction of the fluorescence intensity of the corresponding blank, the concentration read from the calibration curve of serum standards was multiplied by the dilution factor to give the concentration of drug in the serum.

Results and Discussion

The fluorescence excitation and emission spectra of R-HCl in 0.1 N H_2SO_4 are shown in Fig. 2. The excitation spectrum shows three maxima at wavelengths 246, 296.5 and 340 nm with emission at 526 nm. The emission spectrum shows a maximum at 526 nm with excitation at 246, 296 or 340 nm. Although the maximum intensity of fluorescence was at 246 nm, the wavelength of excitation at 340 nm was selected to minimize interferences from serum components.

Different solvent systems were tested for the dilution of the samples. The fluorescence intensities, corrected for the corresponding blank intensity, for R-HCl (1 μg ml^{-1}) were in the following order: 0.1 N H_2SO_4 > H_2O > 0.1 N NaOH > EtOH. The fluorescence intensity reached a maximum value immediately after dilution and remained unchanged for more than 10 days.

The fluorescence intensity was proportional to the concentration of rufloxacin HCl in 0.1 N H_2SO_4 over the range of 0.005–1.5 μg ml^{-1} and over the range 0.01–0.07 μg ml^{-1} for spiked serum diluted with 0.1 N H_2SO_4. The regression equations were $y = 0.45 - 0.73x$ ($n = 15$; $r = 0.9997$) and $y = 1.41 + 0.69x$ ($n = 10$; $r = 0.996$) respectively, where y = the fluorescence intensity and x = concentration of R-HCl in ng ml^{-1}. The detection limit, defined as the sample concentration giving a fluorescence intensity that is three times the standard deviation of the fluorescence intensities of the blank solution [18], was found to be 0.3 ng ml^{-1}. The precision of the proposed method was studied by determining R-HCl 20 times in a standard solution (50 ng ml^{-1}). The standard deviation,

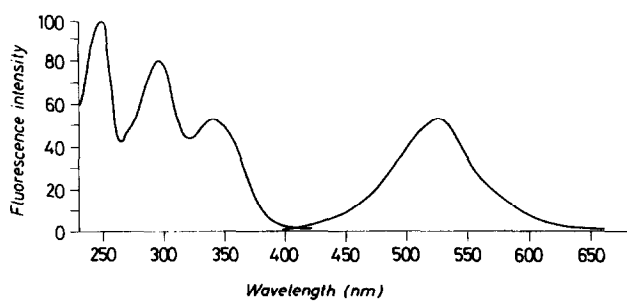

Figure 2

relative standard deviation and relative error were found to be 0.23 ng ml^{-1}, 0.46% and 0.04%, respectively.

The results obtained by analysing capsule formulations containing 100 and 200 mg of drug are shown in Table 1. The recovery of R-HCl was 98–104%. With the experimental conditions used no interference was observed from the water-soluble excipients.

In a preliminary application of this method serum concentrations obtained from one healthy volunteer after oral administration of a single 300 mg dose of R-HCl were assayed (Fig. 3). Maximum drug concentrations which were reached about 2 h after administration, remain constant for a further 10 h and then decrease.

Concentrations in serum obtained from another healthy volunteer after oral administration of doses of 400 mg on the first day and 200 mg on the second and the third days were as follows: 4 h after the first dose the serum concentration was 4.98 ± 0.12 μg ml^{-1} and this decreased to 3.48 ± 0.23 μg ml^{-1} after 12 h. 4 h after the second or the third doses, the concentrations of drug in serum (4.97 ± 0.02 and 5.50 ± 0.28 μg ml^{-1}, respectively) were similar to the value on the first day.

Because the drug shows a strong fluorescence above 500 nm, the background fluorescence of the serum (possibly due to aromatic amino acids especially tryptophan, which fluoresces maximally at about 350 nm when excited at 280 nm) showed only a small contribution which was corrected by subtracting the corresponding blank value

Table 1
Assay of R-HCl in capsules

	R-HCl content			
Sample	mg/cap. declared	μg ml^{-1} found after dilution	mg/cap. found	spectrophotometry mg/cap. found
Capsules	100	0.104 (0.002)*	104	100.5 (0.9)*
		0.049 (0.002)	98	
Capsules	200	0.103 (0.003)*	206	211.3 (1.0)*
		0.0505 (0.004)*	202	

*Mean of five determinations with standard deviation in parentheses.
†Determined at λ_{max} 246 nm in H_2O.

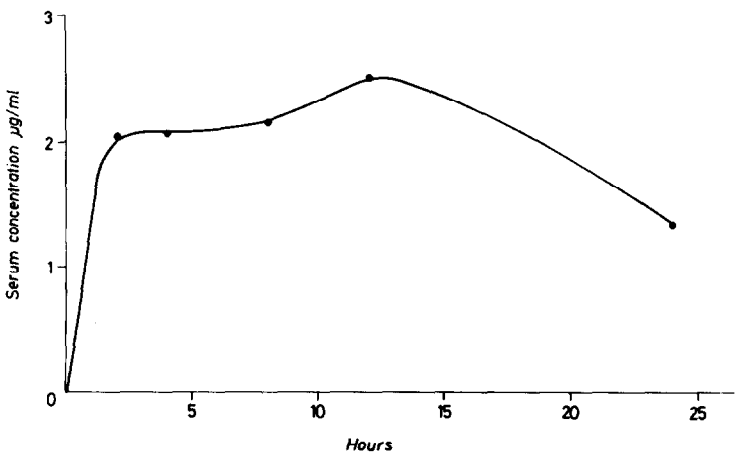

Figure 3

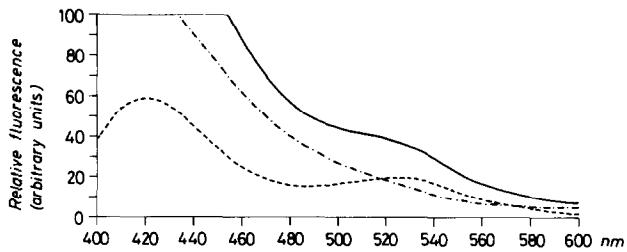

Figure 4

from every reading. Typical fluorescence spectra of rufloxacin HCl in serum before and after subtraction of the corresponding blank value are shown in Fig. 4. The main advantages of the fluorimetric determination of R-HCl are the high sensitivity and the small volumes of serum (0.05 ml or less) needed for the assay. The proposed method can be applied to the determination of serum levels of rufloxacin in pharmacokinetics studies.

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