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Short Communication

The analytical profile of rufloxacin, a new fluoroquinolone, by reversed-phase high-performance liquid chromatography*

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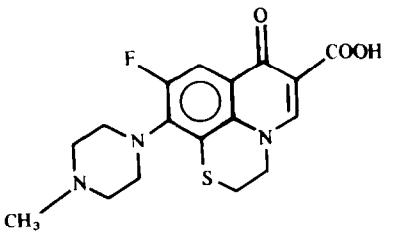
Introduction

Growing interest in quinolones has led to a study aimed at developing a chromatographic method for the determination of the purity profile of bulk drug and pharmaceutical preparations containing rufloxacin, a new synthetic fluoroquinolone. Rufloxacin, 9-fluoro-10-[*N*-(4-methyl)-1-piperazinyl]-7-oxo-2,3-7H-pyrido[1,2,3-*d*,*e*] [1–4] benzothiazine-6-carboxylic acid hydrochloride (**I**), is an antibacterial agent [1–3] which has a specific activity against gram-negative bacteria.

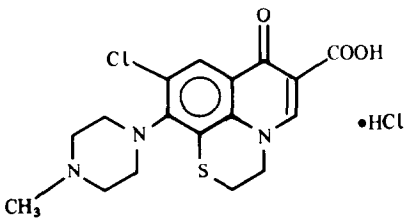


I

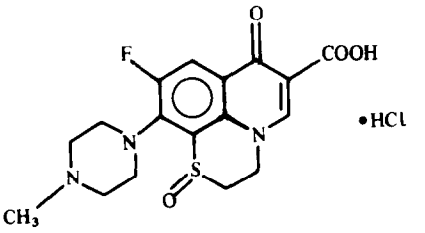
This compound is almost completely absorbed from the gastrointestinal tract after oral administration and shows an activity comparable to that of nalidixic acid, but fewer side effects than other fluoroquinolones.

During the synthesis of rufloxacin two impurities has been identified, 9-fluoro-10-[*N*-(4-methyl)-1-piperazinyl]-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-*d*,*e*] [1–4] benzothiazine-1-oxide-6-carboxylic acid (**II**) and the 9-chlor-10-[*N*-(4-methyl)-1-piperazinyl]-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-*d*,*e*] [1–4] benzothiazine-6-carboxylic acid (**III**).

It is expected that rufloxacin will be approved for marketing within a short time. At



III



II

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present there are no literature methods describing the analysis of this drug and its related compounds, therefore, there is a need to find correct conditions for the qualitative and quantitative analysis of this drug and its impurities in bulk material and in pharmaceutical preparations. In this paper a reversed-phase high-performance liquid chromatography/diode array detection method for the analysis of rufloxacin and related compounds is proposed.

Experimental

Apparatus

An high-performance liquid chromatograph Waters Model 6000 A (Waters Ass., Milford, MA, USA), equipped with a diode array detector HP 1040 M controlled by a computer HP 9000 Model 310 (Hewlett-Packard, Washington, USA), was used in the investigation. Standard and sample solutions were injected via a U6K loop valve injector (Waters Ass.) using a manual method.

Reagents and chemicals

Pure standards of rufloxacin hydrochloride and related compounds, rufloxacin raw material and its pharmaceutical forms (capsules) were kindly supplied by Mediolanum Farmaceutici (Milano, Italy). All other chemicals and solvents, obtained from Merck (Darmstadt, FRG), were of analytical or HPLC grade and were used without further purification.

Chromatographic conditions

The chromatographic separation of rufloxacin and related compounds was achieved by means of a Nucleosil RP-18, 5-μm (250 × 4.6 mm i.d.) column. The eluent was 0.1% acetic acid in 0.1 M trihydrogen orthophosphate–acetonitrile (85:15, v/v) delivered at a flow rate of 1 ml min.

Procedure

Standard solutions. (a) 10 mg of rufloxacin hydrochloride standard was weighed exactly, transferred to a 10-ml volumetric flask and diluted to volume with HPLC water. 1 ml of this solution was transferred to a 20-ml volumetric flask and diluted to volume with the solvent mixture used as eluent in HPLC analysis. (b) 10 mg of each impurity was transferred to a 100 ml volumetric flask and made up to the mark with HPLC water. 1 ml of this solution was transferred to a 100-ml volumetric flask and diluted to volume with the HPLC eluent.

Calibration curves

The calibration curves were obtained by injection of different volumes of standard solutions of compounds **I**, **II** and **III** and plotting the analytical data by means of a computer.

Qualitative and quantitative analysis

A qualitative analysis and the quantitative determination of rufloxacin and its related compounds has been made on three different lots of raw material and capsules containing the drug. Samples for HPLC analyses were prepared as follows.

Raw material. Fifty milligrams of rufloxacin hydrochloride raw material, weighed exactly, was transferred to a 50-ml volumetric flask and diluted up to the mark with water. 5 ml of this solution was put into a 50 ml volumetric flask and diluted to volume with eluent and this solution used for analytical purposes.

Pharmaceuticals. Ten capsules containing 300 mg of rufloxacin hydrochloride were opened, the contents powdered and weighed. The medium weight of one capsule was 565 mg ± 9.42. A weight of powder equivalent to about 500 mg was transferred to a 100-ml volumetric flask, containing about 50 ml of purified water, stirred for 20 min and then diluted to volume with water. This suspension was quantitatively filtered through a paper filter and then through a 0.45-μm Millipore membrane. 20 ml of the solution obtained was diluted to 100 ml with HPLC eluent and this solution was used for the qualitative and quantitative analyses.

The quantitative analyses were carried out using the calibration curves constructed previously.

Results and Discussion

The chromatographic conditions of the proposed method enable the separation of rufloxacin from its impurities **II** and **III**. Figure 1 shows the chromatographic profile obtained for a raw material sample solution. In the chromatogram three peaks, corresponding to

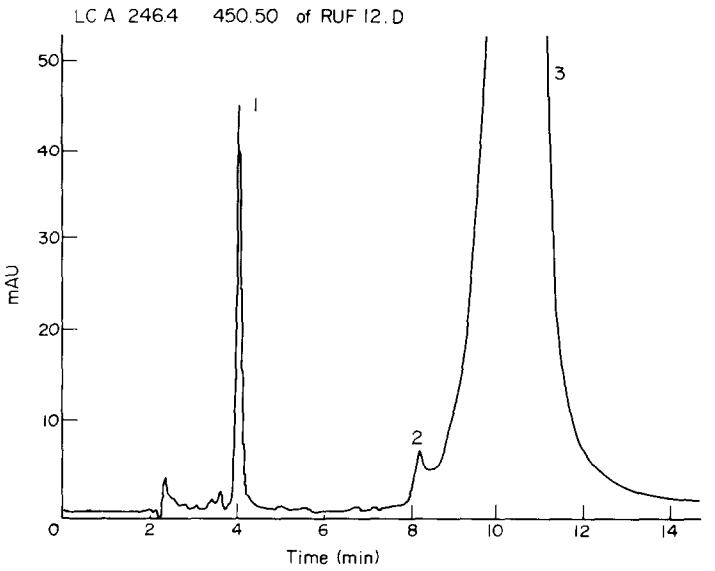

Figure 1

rufloxacin (peak 3), impurities **II** (peak 1) and **III** (peak 2), are present. During the chromatographic run the diode array detector gave for peaks 1 and 3 UV spectra which were identical to those obtained from the chromatographic analyses of the standard solutions of these compounds (Fig. 2). Peak 2 of the chromatogram is very small so the UV spectrum is not very clear, but by addition of a known amount of impurity **III** standard to raw material sample solution the UV spectrum became identical to that of impurity **III** (Fig. 3a). This spectrum is very similar to that of rufloxacin (Fig. 3b) this is to be expected as the difference between the structure of the impurity and rufloxacin is merely substitution of the chlorine for fluorine in the 9 position. All analyses carried out on these pharmaceuticals show the presence of two impurities, especially impurity **II**. Sample solutions of raw material and pharmaceutical preparations need to be prepared daily because the rufloxacin-1-oxide (impurity **II**) increases if the rufloxacin sample remains in the eluent solution presumably due to aerobic oxidation.

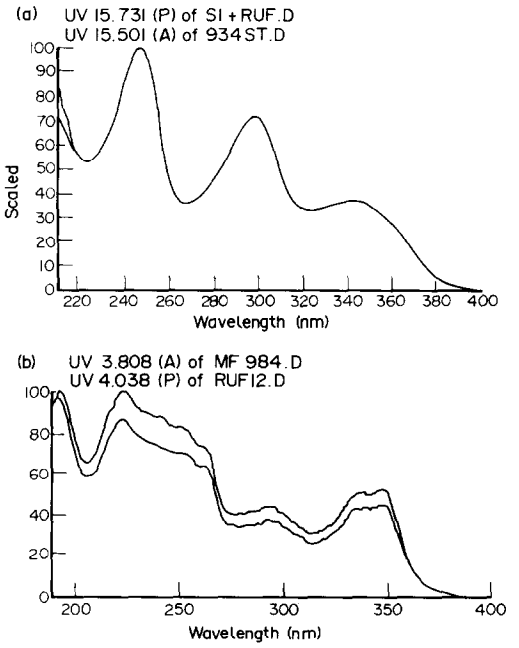

Figure 2

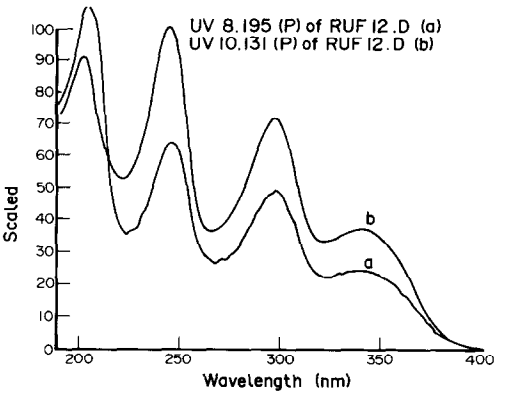

Figure 3

A quantitative analysis was performed using an external standard method. The calibration curves of rufloxacin and impurities **II** and **III** were found to be linear over the range 0.01–

2 mg ml^{-1} for the rufloxacin standard and 0.005 and 0.1 mg ml^{-1} for the two impurities. The correlation coefficients and relative standard deviations, obtained from 10 determinations, were of 0.999 ± 1.3% for the rufloxacin, of 0.989 ± 0.7 and of 0.991 ± 0.9%, respectively, for impurities **II** and **III**. The method was checked using samples prepared by adding known amounts of impurities **II** and **III** to impurity-free rufloxacin hydrochloride. The minimum detectable amount of the two impurities was 5 ng. The quantitative data obtained from the raw material and pharmaceutical analyses impurities are summarized in Table 1.

The proposed method gives a good separation of the rufloxacin from its related compound and repeated analyses on the same samples shows that is reproducible, sensitive and selective.

Table 1
Control analyses of raw material and pharmaceutical samples

	Pharmaceutical form			Raw material		
	I	**II**	**III**	**I**	**II**	**III**
S1	99.25	0.65	0.034	99.28	0.62	0.030
S2	99.07	0.63	0.041	99.30	0.60	0.052
S3	99.19	0.61	0.020	99.35	0.61	0.023

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