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QUANTITATION OF CIPROFLOXACIN HYDROCHLORIDE AND NORFLOXACIN IN TABLETS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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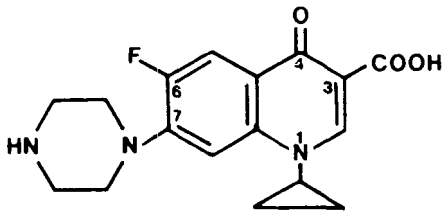
ABSTRACT

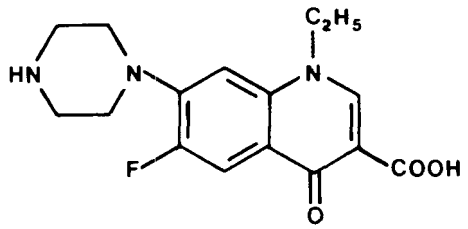
A stability-indicating high-performance liquid chromatography method for the quantitation of ciprofloxacin and norfloxacin in tablets (the only dosage form available at present) has been developed. The method is precise and accurate with a percent relative standard deviation based on 5 readings of 1.2 and 1.4 for ciprofloxacin and norfloxacin, respectively. A number of inactive ingredients present in the tablets did not interfere with the assay procedures. The addition of hydrochloric acid in the extraction procedure was necessary for the quantitative recovery and reproducible results. The recovery from the synthetic mixtures was quantitative. Both the drugs (quinolones) appear to be very stable since 10 minute boiling with either sulfuric acid or sodium hydroxide solution caused very little decomposition.

BACKGROUND

Ciprofloxacin and norfloxacin (Figure 1) are synthetic broad spectrum antibacterial agents for oral administration. Structurally, both of these drugs are fluoroquinolone and similar. They are new antibacterial agents and are extensively used to treat urinary tract



Ciprofloxacin



Norfloxacin

Figure 1 Structures of ciprofloxacin and norfloxacin.

infection. In spite of the wide use of these drugs, very little information is available in the literature concerning the quantitative analysis of the active ingredients. The purpose of these investigations was to develop an HPLC method for the quantitation of ciprofloxacin and norfloxacin in tablets (the only dosage form available at present).

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents were USP-NF or ACS quality and used without further purification. The ciprofloxacin powder was supplied by Miles Laboratories and the norfloxacin powder by Merck Sharp & Dohme. All the tablets were of the commercial lots.

Apparatus: A high-pressure liquid chromatograph (Waters ALC 202) equipped with a universal injector (Rheodyne Model 7125), a multiple

wavelength detector (Schoeffel's SF 770, Kratos Inc.) and a recorder (Omniscribe 5213-12, Houston Instruments) was used. A C_{18} column (Microbondapak by Waters, 30 cm x 3.9 mm i.d) was the stationary phase.

Chromatographic Conditions: The mobile phase contained (by volume) 15% acetonitrile, 12% methanol and 0.3% glacial acetic acid in 0.01M KH_2PO_4 aqueous buffer solution. The flow rate was 2.0 ml, the sensitivity was set at 0.04 AUFS (276 nm), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Solutions: The stock solutions were prepared by mixing a 30.0 mg quantity of the ciprofloxacin powder (21.0 mg of norfloxacin powder), with 1 ml of ∿1N HCl and bringing the mixture to volume (100.0 ml) with water. A 225 mg of cephalothin powder (Keflin® by E. Lilly & Co.) was dissolved in enough water to make 100.0 ml of the solution. This stock solution was used as the internal standard for both of the drugs. The stock solutions were diluted further with water as needed. The most commonly used standard solution of ciprofloxacin contained 12.0 µg/ml of the drug and 225 µg/ml of cephalothin. The standard solution of norfloxacin contained 8.4 µg/ml of the drug and 180 µg/ml of cephalothin (the internal standard).

Extraction Procedure From The Tablets: Ten tablets were accurately weighed and ground to a fine powder. A quantity of the powder representing 30.0 mg of ciprofloxacin base (21.0 mg of norfloxacin base) based on the label claim was accurately weighed. The powder was mixed with 1 ml of 1N HCl and stirred with 2 minutes in a 150 ml beaker. The mixture was brought to 100 ml (in a volumetric flask) with water. It was filtered (Fisher's 9-803-5E filter paper), first 15 ml of the filtrate was rejected and then the clear filtrate was collected for further dilution with water. A 2.0 ml quantity of the filtrate was

mixed with a 5.0 ml quantity (4.0 ml for norfloxacin) of the stock solution of cephalothin (the internal standard) and brought to 50 ml (in a volumetric flask) with water.

Decomposition of Drugs Under Drastic Conditions: A 2.0 ml quantity of the stock solution of each drug (separately) was mixed with 15 ml of water and either 1 ml of ∿1N H_2SO_4 or 1 ml of ∿1N NaOH solution in a 150 ml beaker. The mixture was heated to boiling using a hot plate for 10 minutes (more water was added as needed), cooled and neutralized using either ∿1N H_2SO_4 or ∿1N NaOH solution. It was then brought to volume (50.0 ml) with water and assayed using the developed HPLC procedure. No internal standard was added in order to detect any new peaks from the decomposition products.

Assay Procedure: A 20.0 µl quantity of the assay solution was injected into the chromatograph using the conditions described. For comparison, an identical quantity of the standard solution was injected after the assay sample eluted. The standard solution contained identical concentrations of the drug (based on the label claim) and the internal standard.

Calculations: Preliminary investigations indicated that the ratio of the peak heights (drug/internal standard) were directly related to the concentrations of the drug (range tested ± 25% of the standard concentration). Therefore, the results were calculated using a simple equation:

$$\frac{(R_{ph})_a}{(R_{ph})_s} \times 100 = \text{percent of the label claim found}$$

where $(R_{ph})_a$ is the ratio of the peak heights of the assay solution and $(R_{ph})_s$ that of the standard solution. In the case of the decom-

TABLE 1

ASSAY RESULTS OF VARIOUS DOSAGE FORMS AND SYNTHETIC MIXTURES

Drug	Claim per Tablet	Percent of the Label Claim Found
Ciprofloxacin	250 mg	99.5
Ciprofloxacin (different lot)	250 mg	100.2
Ciprofloxacin	500 mg	98.6
Ciprofloxacin (different lot)	500 mg	99.1
Ciprofloxacin	750 mg	100.3
Ciprofloxacin (different lot)	750 mg	99.2
Ciprofloxacin Synthetic Mixture 1	30 mg + 50 mg lactose	100.5
Ciprofloxacin Synthetic Mixture 2	30 mg + 50 mg dextrose	100.2
Norfloxacin	400 mg	99.0
Norfloxacin (different lot)	400 mg	99.2
Norfloxacin Synthetic Mixture 1	21 mg + 50 mg lactose	100.9
Norfloxacin Synthetic Mixture #1	21 mg + 50 mg dextrose	100.8

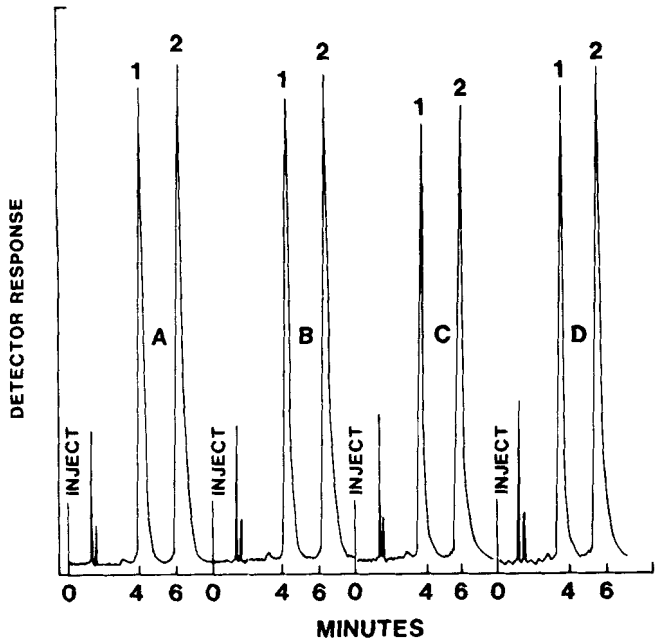


Figure 2 Sample chromatograms. Peak 1 in chromatograms A-B is from ciprofloxacin and in C-D from norfloxacin. Peak 2 is from cephalothin (the internal standard). Chromatogram A is from a standard solution of ciprofloxacin; B from ciprofloxacin 500 mg tablets; C from a standard solution of norfloxacin and D from norfloxacin 400 mg tablets. For chromatographic conditions, see text.

posed solutions without the internal standard, the results were estimated by the direct comparison of drug peak heights (assay/standard) and then multiplying by 100.

RESULTS AND DISCUSSION

The results (Table 1) indicate that the developed HPLC method can be used to quantify ciprofloxacin and norfloxacin in tablets, the only

dosage form available at present. The method is accurate and precise with a percent relative standard deviations based on 5 readings of 1.2 and 1.4 for ciprofloxacin and norfloxacin, respectively. The separation of each drug from the internal standard was complete (Figure 2). Both drugs (quinolones) appear to be very stable since the samples when boiled for 10 minutes either with sulfuric acid or sodium hydroxide (see text) showed very little loss in the potency. No new peaks were detected in the chromatograms. An earlier study reported (1) a 10% loss in potency of norfloxacin in 83 days at pH 5.5 and an ionic strength of 0.2. The study also reported that an increase in the ionic strength increased the rate of degradation. Hence a 0.2 ionic strength value may have caused this degradation. Furthermore, the analysis was conducted using a tedious extraction procedure and subsequent measurement of the absorption value at 270 nm using a spectrophotometer. These investigations (1) were conducted only to determine the effect of ionic strength on the stability of norfloxacin at pH 5.5 and high temperatures. The data was then extrapolated to determine the K_{obs} value at room temperature.

Extraction Procedure: Preliminary investigations indicated that ciprofloxacin hydrochloride powder was instantaneously soluble in water while norfloxacin powder did not dissolve (21 mg/100 ml) without the addition of hydrochloric acid. Therefore, the addition of hydrochloric acid was necessary for the extraction of norfloxacin. The extraction of ciprofloxacin (present as hydrochloride salt in tablets) from the tablets without the addition of hydrochloric acid gave 32-87% of the results (based on the label claim). The results were not reproducible. The addition of hydrochloric acid to both the standard and assay solutions gave quantitative and reproducible results. The inactive ingre-

dients, cellulose, croscarmellose sodium, crospovidone, hydroxypropyl cellulose, hydroxypropyl methylcellulose, iron oxide, magnesium stearate, polyethylene glycol, silicon dioxide, starch and titanium oxide present in the tablets did not interfere with the extraction and the assay procedures. The recoveries from the synthetic mixtures for both ciprofloxacin and norfloxacin were quantitative (Table 1).

REFERENCE

1. G.N. Singh, R.P. Gupta and P. Prakash, Die Pharmazie, 43 (2), 134 (1988).