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A High Performance Liquid Chromatographic Procedure for the Simultaneous Determination of Norfloxacin and Furprofen in Rat Plasma

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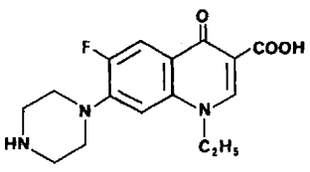
A rapid and simple high performance liquid chromatographic analytical method is described for the simultaneous determination of norfloxacin and furprofen in rat plasma. Following dichloromethane extraction, the solution was chromatographed in a Vydac anion exchange column using a mobile phase of 0.05 M phosphate buffer (pH = 7.0):acetonitrile (80:20, v/v) at a flow-rate of 1.8 mL/min. The drugs were detected by UV absorption at 278 nm. The total chromatographic analysis time was 10 min. The response was linear, 0.1–5.0 μg/mL for norfloxacin and 0.1–3.0 μg/mL for furprofen, respectively. This method is useful for pharmacokinetic studies of these compounds and will facilitate detailed investigations into the interactions between quinolones and furprofen.

INTRODUCTION

Norfloxacin (**1**), 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, is a quinolone antibacterial agent with a broad spectrum of activity (Khan *et al.*, 1982; Muytjens *et al.*, 1983) and widely used in the treatment of urinary tract infections (Kumamoto and Ishigami, 1983; Bologna *et al.*, 1983). Furprofen (**2**), 2-[4-(2′-furoyl)phenyl]propionic acid, is a new non-steroidal anti-inflammatory drug with analgesic properties, active by the inhibition of prostaglandin synthesis (Palumbo *et al.*, 1991). Recently, it was reported that the concomitant administration of a new quinolone antibacterial agent and a non-steroidal anti-inflammatory agent induced convulsions in several cases (Drug Adverse Reaction Information, Ministry of Health and Welfare, Tokyo, 1986, No. 81). The interest in this group of compounds has prompted us to develop a simple, selective and sensitive assay method for these drugs in biological fluids, as there is no adequate method for determining them either simultaneously or readily. In this work we have developed a specific method for the simultaneous determination of norfloxacin and furprofen in rat plasma by high performance liquid chromatography (HPLC).



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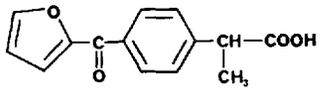

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EXPERIMENTAL

Chemicals and reagents. Furprofen was supplied by the Department of Pharmacology of the University of L'Aquila. Norfloxacin was supplied by Merck Sharp & Dohme Italia (Rome, Italy). Sodium hydroxide and all other chemicals (analytical grade) were obtained from Fluka Chemie (Buchs, Switzerland). HPLC grade acetonitrile was purchased from Farmitalia-Carlo Erba (Milan, Italy). Water was purified and deionized using a Milli-Q ion exchange filtration system (Millipore, Bedford, MA, USA). Water was filtered through WCN 0.45 μm filters, while acetonitrile was filtered through WTP 0.5 μm filters (Whatman, Maidstone, UK).

Chromatographic system and conditions. The chromatographic apparatus (Waters Assoc., Milford, MA, USA) consisted of a Model 510 solvent delivery system, and a Model 484 spectrophotometric detector connected to a Model HP-3396-II integrator (Hewlett-Packard, Rome, Italy). A Model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 20 μl loop was also used.

The separation was performed on an anion exchange Vydac (250 × 4.6 mm, 10 μm particle size) column (Separation Group, Hesperia, CA, USA), connected to an anion exchange AXGU (20 × 4.6 mm, 10 μm particle size) precolumn (Brownlee Labs Inc., Santa Clara, CA, USA). The mobile phase consisted of a mixture of 0.05 M phosphate buffer (pH 7.0):acetonitrile (80:20, v/v). The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow-rate of 1.8 mL/min. The detector wavelength was set at 278 nm.

Animals. Sprague-Dawley male and female albino rats, weighing 250–300 g, were housed at 21 ± 1 °C with a humidity range of 40–60% and maintained on a 12 h light–dark cycle. Drugs were administered orally in a 0.5% suspension with

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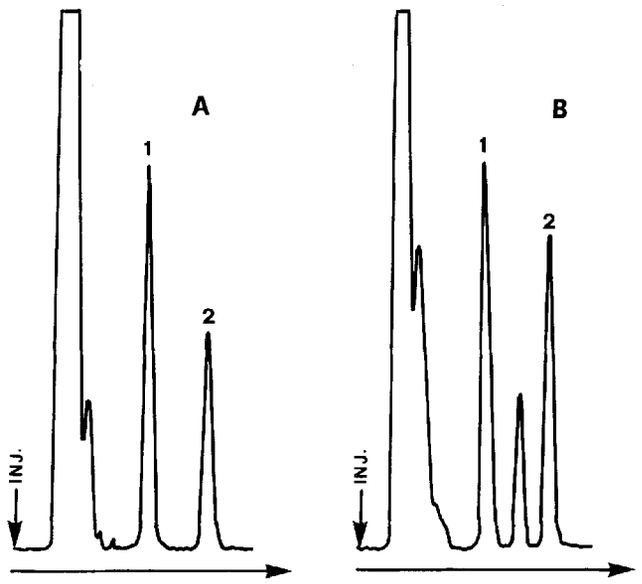

Figure 1. Chromatograms from rat plasma extract: (A) blank plasma spiked with 2.5 μg/mL of furprofen (1) and 2.5 μg/mL of norfloxacin (2); (B) plasma sample taken two hours after administration of the drugs.

Table 1. Within-day precision and accuracy in the calibration standard of norfloxacin in rat plasma

Theoretical concentration (μg/mL)	Measured concentration[a] (μg/mL)	CV (%)	RE (%)
0.1	0.096±0.007	7.2	4.1
0.2	0.19±0.01	5.2	5.2
0.3	0.29±0.01	3.4	3.4
0.5	0.48±0.02	4.1	4.1
1.0	0.96±0.03	3.1	4.1
2.0	1.90±0.05	2.0	5.2
3.0	2.88±0.06	2.0	4.1
5.0	4.88±0.09	1.8	2.4

[a] Mean of five assays±standard deviation.

carboxymethylcellulose at neutral pH and at a dose of 10 mg/Kg (norfloxacin) and 2 mg/Kg (furprofen). The blood samples were taken after two hours.

Extraction of plasma samples. 0.5 mL of plasma was added to 100 μL of 0.05 M NaOH and 2 mL of CH_2Cl_2 in a centrifuge tube, shaken for 5 min and centrifuged at 1500 × g for 10 min. The extraction procedure was repeated twice. The organic phases collected were evaporated to dryness under vacuum and the residue was added to 100 μL of 0.05 M NaOH. Aliquots of 20 μL were chromatographed.

Standard solutions and calibration curves. A stock solution (1 mg/mL) of norfloxacin and furprofen was prepared in 0.05 M NaOH. Standard solutions were prepared with control rat plasma in the concentration range of 0.1–5 μg/mL (norfloxacin) and 0.1–3 μg/mL (furprofen), maintaining the concentration of the other drug at a constant level of 2 μg/mL. The calibration curves were obtained by plotting the peak-area of each drug extracted versus its concentration.

RESULTS AND DISCUSSION

Figure 1 illustrates typical chromatograms obtained from rat plasma extracts; they do not contain any interfering peak or endogenous component with a retention time similar to that of norfloxacin (5.3 min) and furprofen (4.7 min).

Table 2. Between-day precision and accuracy in the calibration standard of norfloxacin in rat plasma

Theoretical concentration (μg/mL)	Measured concentration[a] (μg/mL)	CV (%)	RE (%)
0.1	0.097±0.009	9.5	3.0
0.2	0.19±0.01	5.8	5.2
0.3	0.29±0.02	6.8	3.4
0.5	0.49±0.01	2.0	2.0
1.0	0.98±0.03	3.0	2.0
2.0	1.90±0.06	3.1	5.2
3.0	2.91±0.08	2.7	3.0
5.0	4.90±0.08	1.6	2.0

[a] Mean of five assays±standard deviation.

Table 3. Within-day precision and accuracy in the calibration standard of furprofen in rat plasma

Theoretical concentration (μg/mL)	Measured concentration[a] (μg/mL)	CV (%)	RE (%)
0.1	0.097±0.006	6.1	3.0
0.2	0.19±0.008	4.2	5.2
0.3	0.29±0.02	6.8	3.4
0.5	0.48±0.02	4.1	4.1
1.0	0.99±0.04	4.0	1.0
2.0	1.97±0.08	4.0	1.5
3.0	2.90±0.07	2.4	3.4

[a] Mean of five assays±standard deviation.

Table 4. Between-day precision and accuracy in the calibration standard of furprofen in rat plasma

Theoretical concentration (μg/mL)	Measured concentration[a] (μg/mL)	CV (%)	RE (%)
0.1	0.095±0.008	8.4	5.2
0.2	0.187±0.01	5.3	6.9
0.3	0.28±0.02	7.1	7.1
0.5	0.47±0.02	4.2	6.3
1.0	0.96±0.03	3.1	4.1
2.0	1.90±0.06	3.1	5.2
3.0	2.87±0.09	3.1	4.5

[a] Mean of five assays±standard deviation.

The calibration curves for the two drugs in rat plasma were linear over the range 0.1–5.0 μg/mL for norfloxacin and 0.1–3.0 μg/mL for furprofen and the correlation coefficients of the calibration curves were 0.999 and 0.997, respectively. The lower detection limit of the method, defined as three times the level of the baseline noise, was 0.05 μg/mL for each drug. Reproducibilities for within-day and between-day assay were evaluated to assess the precision and accuracy of this analytical method. The results are shown in Tables 1–4. The coefficients of variation (CVs) of the five independent samples at each concentration in the within-day assay were between 7.2 and 1.8% with relative errors (REs) of 5.2–2.4 (Table 1) for norfloxacin in the concentration range 0.1–5.0 μg/mL, and in the between-day assay were 9.5 and 1.6% with relative errors of 5.2–2.0%

(Table 2). For furprofen in the concentration range of 0.1–3.0 μg/mL, the CV values in the within-day assay were 6.8 and 2.4% with relative errors of 5.2–1.0% (Table 3) and in the between-day assay were 8.4 and 3.1% with relative errors of 7.1–4.1% (Table 4).

CONCLUSION

We have developed a simple, sensitive, precise and accurate HPLC method for the simultaneous determination of norfloxacin and furprofen in rat plasma. This method is useful for pharmacokinetic studies of these compounds and will facilitate detailed investigations into the interactions between new quinolones and furprofen.

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REFERENCES

Bologna, M., Vaggi, L., Tomei, E. and Martini, E. (1983). *Eur. J. Chemother. Antibiot.* **3**, 41.

Khan, M. Y., Gruninger, R. P., Nelson, S. M. and Ckliker, R. E. (1982). *Antimicrob. Agents Chemother.* **21**, 848.

Kumamoto, Y. and Ishigami, J. (1983). *Eur. J. Chemother. Antibiot.* **3**. 31.

Muytjens, H. L., van der Ros-van der Repe, J. and van Veldhnizen, G. (1983). *Antimicrob. Agents Chemother.* **24**, 302.

Palumbo, G., Fanini, D., Pantaleoni, G. C. and Carlucci, G. (1991). *Int. J. Immunopath. Pharmacol.* **4**, 85.