Simultaneous Determination of Furprofen and Rufloxacin in Human Plasma by High-Performance Liquid Chromatography

Giuseppe Carlucci* and Pietro Mazzeo
Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università dell’Aquila, Via Vetoio, 67010 Coppito (L’Aquila), Italy

Abstract
A simple and reproducible method for the simultaneous determination of the nonsteroidal anti-inflammatory agent, furprofen, and the quinolone antimicrobial agent, rufloxacin, in human plasma is described. It involves a two-step liquid-liquid extraction and a separation using an LC-SAX column with ultraviolet detection at 280 nm. Fenbufen is used as the internal standard. Within-day and between-day coefficients of variation are less than 6%. The lower limits of detection are 0.05 and 0.03 µg/mL for furprofen and rufloxacin, respectively. The method is suitable for pharmacological, toxicological, and pharmacokinetic studies of furprofen and rufloxacin.

Introduction
Furprofen, or 2-[4-(2'-furoyl)phenyl]propionic acid (Figure 1A), is a potent anti-inflammatory drug with analgesic properties active by inhibition of prostaglandin synthesis (1). Rufloxacin is a new long-acting, once-daily quinolone antibacterial; it is highly active in vitro against a broad spectrum of Gram-positive and Gram-negative organisms, including those resistant to β-lactam antibiotics (2,3). Its chemical name is 9-fluoro-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido [1,2,3-de][1,4]benzothiazine-6-carboxylic acid hydrochloride. Its formula is shown in Figure 1B. However, a number of authors have shown that certain quinolones have a pharmacokinetic interaction with nonsteroidal anti-inflammatory drugs (NSAIDs) due to metabolic inhibition (4). Recently, it was reported that concomitant administration of a new quinolone antibacterial agent and a nonsteroidal anti-inflammatory agent induced convulsions in several cases (5). So far, no method has been described for the simultaneous determination of these compounds in biological fluids.

The interest in this group of drugs has prompted us to develop a simple and sensitive assay method for both these substances in human plasma, which could be applied to pharmacokinetic studies. The procedure, which is based on the use of high-performance liquid chromatography (HPLC), allows accurate and precise results.

Experimental

Equipment
HPLC analysis was carried out using a Waters system (Milford, MA) composed of the following: a Waters Model 510 pump, a Lambda Max Model 481 LC variable wavelength detector connected to a Model HP-3396-II integrator (Hewlett-Packard; Rome, Italy). A Model 7125 sample injector (Rheodyne; Cotati, CA) equipped with a 20-µL loop was also used. The separation system was an anion-exchange Supelcosil LC-SAX 5-µm analytical column (25 cm × 4.6-mm i.d.) (Supelco; Bellefonte, PA) protected by an anion-exchange Vydac AXGU 2-cm × 4.6-mm i.d. (10 µm) precolumn (Separation Group; Hesperia, CA). Both were used at ambient temperature.

* Author to whom correspondence should be addressed.

Figure 1. Chemical structures of furprofen (A), rufloxacin (B), and fenbufen (C).
Chemicals and reagents

HPLC-grade acetonitrile, dichloromethane, and all other analytical-grade reagents (sodium hydroxide, sodium hydrogen phosphate, and potassium dihydrogen phosphate) were obtained from Farmitalia Carlo Erba (Rome, Italy). Water was purified and deionized using a Milli-Q ion-exchange filtration system (Millipore; Bedford, MA). Phosphate buffer was filtered through WCN 0.45-µm filters, and acetonitrile was filtered through WTP 0.5-µm filters (Whatman; Maidstone, UK). Rufloxacin and furprofen were supplied by the Department of Pharmacology of this university. Fenbufen was purchased from Aldrich Italia (Milan, Italy).

Calibration curve

Stock solutions of furprofen (1 mg/mL), rufloxacin (2 mg/mL), and fenbufen (100 µg/mL) were prepared in 0.02M sodium hydroxide. Dilutions of these solutions were made in order to prepare the plasma standards needed to construct the calibration curves. Fenbufen was used as the internal standard. Retention times were determined by injecting an aliquot of the standard solution into the HPLC system. Plasma standards were prepared by adding aliquots of the respective stock solutions of furprofen and rufloxacin to give seven appropriate final concentrations in drug-free human plasma. Plasma samples for furprofen were 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, and 10.0 µg/mL. Rufloxacin concentrations were 0.1, 0.2, 1.0, 2.0, 3.0, 5.0, and 10.0 µg/mL. Calibration graphs were obtained by plotting the peak-height ratios for each compound to that of the internal standard against concentration. Linear calibration graphs were obtained.

Extraction procedure

Furprofen, rufloxacin, and fenbufen were extracted from plasma using a simple two-step extraction procedure. An aliquot (20 µL) of the internal standard stock solution (100 µg/mL) was added to 1.0 mL of plasma. Phosphate buffer (0.5 mL of 0.05M) at pH 7 was then added, and the mixture was vortex mixed for 1 min. Dichloromethane (2 mL) was added, and the tubes were vortex mixed for 1 min and shaken for 5 min. The samples were centrifuged at 100 × g for 10 min, and the organic layer was separated. The extraction procedure was repeated twice. The organic phases thus collected were evaporated to dryness under vacuum, and the residue was added to 200 µL of 0.02M NaOH. For HPLC analysis, a portion (20 µL) of the solution was injected.

Chromatographic conditions

Each sample was analyzed by ultraviolet detection at 280 nm. The mobile phase consisted of a mixture of acetonitrile–0.05M phosphate buffer (pH 7.0) (10:90 v/v). The mobile phase was prepared daily, sonicated before use, and delivered at a flow rate of 1.5 mL/min, which produced a column pressure of 16 MPa. The chromatogram from this system is shown in Figure 2.

Accuracy and precision

To test the accuracy and precision of this assay, plasma samples containing 0.2 and 2.0 µg/mL furprofen and plasma samples containing 0.2 and 3.0 µg/mL rufloxacin were prepared. The within-day (n = 10) and between-day (n = 10) variations were evaluated by repetitive analysis of these spiked plasma samples. The amount of drug in these samples, as well as the unknown, were obtained through linear regression analysis.

Lower limits of detection

Lower limits of detection, which were defined as a signal-to-noise ratio of 3, were determined using aqueous standard solutions.

Percent extraction efficiency

Percent extraction efficiencies were determined by comparing the peak height ratios of chromatograms obtained from extracted plasma samples with those of the standard.
aqueous solutions at concentrations of 1.0 and 2.0 µg/mL of furprofen and rufloxacin, respectively (n = 3).

Results and Discussion

Figure 2 shows a typical chromatogram of an extracted drug-free plasma blank (A), an extracted plasma sample (B), and a sample obtained from a volunteer 2 h after administration (C). The elution order is fenbufen, furprofen, and rufloxacin. No endogenous plasma components or metabolites were observed near the retention times corresponding to furprofen or rufloxacin. The accuracy and precision of the assay are shown in Table I. Analysis of the seven standards revealed that the curves for furprofen and rufloxacin were linear over the concentration ranges examined. All correlation coefficients were more than 0.9997. The straight line equations for furprofen and rufloxacin were $y = (0.115)C - 1.44$ and $y = (0.121)C - 0.212$, respectively, where $y$ is the peak height ratio and $C$ is concentration in micrograms per milliliter. From these equations, the concentrations of the analytes were determined. The method was found to be reproducible and accurate. The high and low values had coefficients of variation that were less than 6% (Table I). Mean extraction efficiencies were 92.3, 97, and 94% for furprofen, fenbufen, and rufloxacin, respectively. Limits of detection for fenbufen, furprofen, and rufloxacin were calculated to be 0.02, 0.05, and 0.03 µg/mL, respectively. Comparison of peak height ratios for furprofen and rufloxacin from fresh plasma samples (2.0 µg/mL and 3.0 µg/mL) with the ratios from samples frozen at −20°C for a period of 2 weeks showed no differences. Retention times for fenbufen, furprofen, and rufloxacin were 3.5, 4.9, and 7.3 min, respectively.

Conclusion

We have developed a simple, sensitive, precise, and accurate HPLC method for the simultaneous determination of furprofen and rufloxacin in 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


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