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Simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography

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Abstract

A simple, specific and sensitive high-performance liquid chromatographic method has been developed for the simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma. Plasma, spiked with internal standard, was vortex-mixed for 1 min with a mixture of dichloromethane–diethyl ether (80:20, v/v). The evaporated extract was dissolved in 0.02 *M* NaOH. Drugs were resolved at room temperature on a 5 μm Zorbax SAX column (250×4.6 mm I.D.) equipped with a 20×4.6 mm anion-exchange Vydac AXGU (10 μm particle size) precolumn. The mobile phase consisted of acetonitrile and phosphate buffer (pH 7.0), delivered at a flow-rate of 1.2 ml/min. Detection was made at 280 nm. 2-[4-(2′-Furoyl)phenyl]propionic acid was used as internal standard. The calibration curve was linear from 0.2 to 10 μg/ml for rufloxacin, from 0.5 to 30 μg/ml for fenbufen and from 0.2 to 10 μg/ml for felbinac, respectively. The detection limit was 0.1 μg/ml for rufloxacin, 0.3 μg/ml for fenbufen and 0.1 μg/ml for felbinac, respectively.

Keywords: Rufloxacin; Fenbufen; Felbinac

1. Introduction

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 [1,2]. 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 Fig. 1A. Fenbufen, or γ-oxo-[1,1′-biphenyl]-4-butanoic acid (Fig. 1B), is one of the non-steroidal anti-inflammatory, antipyretic and analgesic agents that belongs to the group of propionic acid derivatives; it has been frequently used because of its relative low gastric toxicity [3,4]. This drug is readily transformed in its metabolite biphenylacetate, felbinac (Fig. 1C) [3,4].

In the therapy of infectious disease with inflammation, the quinolones and fenbufen have been routinely co-administered. All the quinolones inhibit the GABA receptors binding to the synaptic membranes in a dose dependent manner [5]. It was reported that some quinolones induced severe convulsions [6]. Fenbufen, but more so its metabolite felbinac, showed a marked intensification of this neurotoxic effect.

Several analytical HPLC methods have been re-

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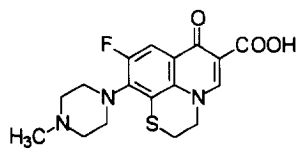

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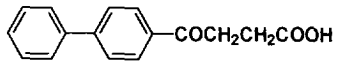

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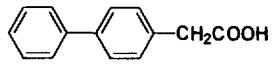

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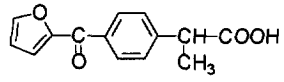

D

Fig. 1. Chemical structures of rufloxacin (A), fenbufen (B), felbinac (C) and internal standard (D).

ported for assaying rufloxacin [7,8] and fenbufen [9,10]. So far no method available for the simultaneous determination of these compounds in biological fluids has been described.

The interest in this group of drugs has prompted us to develop a simple and sensitive assay method for simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma. The procedure, based on the use of the high-performance liquid chromatography, allows accurate and precise results.

2. Experimental

2.1. Chemicals and reagents

Rufloxacin hydrochloride was kindly supplied by Mediolanum Farmaceutici (Milan, Italy). 2-[4-(2′-Furoyl)phenyl]propionic acid was prepared in our laboratory and used as internal standard (Fig. 1D). Fenbufen was purchased from Sigma (St. Louis, MO, USA). Felbinac or 4-biphenylacetic acid was purchased from Aldrich Chimica (Milan, Italy). Acetonitrile (HPLC grade), dichloromethane, diethylether and all other analytical-grade reagents (sodium hydroxide, sodium hydrogenphosphate and potassium dihydrogenphosphate) were obtained from Farmitalia-Carlo Erba (Milan, Italy).

2.2. Chromatographic system and conditions

HPLC analysis was carried out using a Waters Associates (Milford, MA, USA) system composed of the following: a Model 510 pump, a Model 486 LC variable-wavelength absorbance 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 used. The analysis was performed on an analytical 250×4.6 mm I.D. anion-exchange Zorbax SAX (5 μm particle size) column (Rockland Technologies, Newport, DE, USA), protected by a 20×4.6 mm I.D. anion-exchange Vydac AXGU (10 μm particle size) guard column (Separations Group, Hesperia, CA, USA). Separations were performed at room temperature. The mobile phase consisted of acetonitrile–0.1 *M* phosphate buffer pH 7.0 (10:90, v/v). Phosphate buffer was filtered through an HA 0.45 μm filter, while the acetonitrile was filtered through a FA 0.5 μm filter (Millipore, Bedford, MA, USA). The mobile phase was prepared daily and delivered at a flow-rate of 1.2 ml/min. Column eluate was monitored at 280 nm.

2.3. Standard solutions and calibration curves

Stock solutions of rufloxacin, fenbufen and felbinac were prepared by dissolving 10 mg of each compound in 10 ml of 0.02 *M* sodium hydroxide. The stock solution of the internal standard was prepared by dissolving 20 mg of compound in 10 ml of methanol; the solution obtained was diluted with methanol as to obtain a concentration of internal standard equivalent to 250 μg/ml. Standard solutions, each containing the three drugs, were prepared with control human plasma in the concentration range of 0.2–10 μg/ml for rufloxacin, 0.5–30 μg/ml for fenbufen and 0.2–10 μg/ml for felbinac. For each solution, the concentration of the other two drugs was kept constant at 5 μg/ml; changing this

concentration to 10 μg/ml was without any effect in each case. An aliquot of the internal standard stock solution was added to each sample, so as to give a concentration of the internal standard equal to 5 μg/ml. The standards were treated concurrently in the same manner as the samples to be analysed. The calibration curves were obtained by plotting the peak-area ratios of each drug to internal standard versus its concentration, obtained after extraction.

2.4. Sample preparation

To an aliquot of plasma (1.0 ml), after thawing, we added the internal standard solution (20 μl) and 100 μl of 0.02 *M* sodium hydroxide, with brief vortex-mixing. The sample was then extracted with 4 ml of a mixture of dichloromethane–diethyl ether (80:20, v/v) and tumbled for 10 min on a rotary mixer. The separation of the two phases was achieved by centrifugation at 2500 *g* for 10 min. The organic phase was separated, transferred into a second tube and evaporated to dryness under a gentle stream of nitrogen at room temperature. The dry residue was then reconstituted with 200 μl of 0.02 *M* NaOH and transferred to chromatographic vials for analysis. 20 μl of these solution were injected onto the chromatographic system.

2.5. Validation of the method

The precision and accuracy of the method were determined by preparing pools of plasma containing rufloxacin, fenbufen and felbinac at nine different concentrations. The values for rufloxacin, fenbufen and felbinac for each standard concentration were determined by nine repeated analyses, using spiked human plasma. Results are given in Table 1, Table 2 and Table 3. The method was found to be reproducible and accurate. The coefficients of variation were less than 4.8% for rufloxacin (Table 1), 4.6% for fenbufen (Table 2) and 4.7% for felbinac (Table 3), respectively. The mean extraction efficiencies calculated by comparison of the peak-area ratios of the extracted samples with those of aqueous standards of same concentration were 95, 96 and 95% for rufloxacin, fenbufen and felbinac. The extraction efficiency of the internal standard was 97%. Data obtained in different days were invariably found to be in good agreement.

Table 1
Precision and accuracy of rufloxacin calibration standards in human plasma

Concentration (μg/ml)	*n*	Concentration found (mean±S.D.) (μg/ml)	C.V. (%)	R.E. (%)
0.20	9	0.19±0.01	4.2	5.2
0.30	9	0.29±0.01	4.8	3.4
0.50	9	0.48±0.02	2.3	3.9
1.00	9	0.98±0.05	4.5	2.0
2.00	9	1.95±0.08	4.1	2.5
5.00	9	4.85±0.10	2.0	3.0
10.00	9	9.83±0.20	2.0	1.7

S.D., standard deviation. C.V., coefficient of variation. R.E., relative error.

Table 2
Precision and accuracy of fenbufen calibration standards in human plasma

Concentration (μg/ml)	*n*	Concentration found (mean±S.D.) (μg/ml)	C.V. (%)	R.E. (%)
0.50	9	0.49±0.02	4.0	2.0
1.00	9	0.96±0.04	3.9	4.1
2.00	9	1.93±0.09	4.6	3.6
5.00	9	4.90±0.16	3.2	2.0
10.00	9	9.90±0.20	2.0	1.0
20.00	9	19.80±0.30	1.5	1.0
30.00	9	29.20±0.50	1.7	2.7

S.D., standard deviation. C.V., coefficient of variation. R.E., relative error.

Table 3
Precision and accuracy of felbinac calibration standards in human plasma

Concentration (μg/ml)	*n*	Concentration found (mean±S.D.) (μg/ml)	C.V. (%)	R.E. (%)
0.20	9	0.19±0.01	4.7	5.2
0.50	9	0.49±0.02	4.0	2.0
1.00	9	0.97±0.02	2.3	3.0
2.00	9	1.97±0.06	2.9	1.5
3.00	9	2.95±0.09	3.0	1.6
5.00	9	4.90±0.11	2.2	2.0
10.00	9	9.85±0.18	1.8	1.5

S.D., standard deviation. C.V., coefficient of variation. R.E., relative error.

3. Results and discussion

The procedure for the simultaneous analysis of fenbufen, rufloxacin and felbinac in human plasma required a one step extraction with dichloromethane-diethylether. The retention times for rufloxacin, fenbufen and felbinac were 5.5, 6.3 and 9.2 min, respectively. The retention time for the internal standard was 3.9 min. Fig. 2 shows a typical chromatogram of a drug-free plasma (A), a spiked plasma sample (B), and a 2-h after-dose sample from a volunteer (C). No interfering peak was present in chromatograms of blanks at the retention times of rufloxacin, fenbufen, felbinac and internal standard. The validity of the liquid chromatographic assay was established through a confirmatory study of calibration curves, specificity, sensitivity, accuracy and precision.

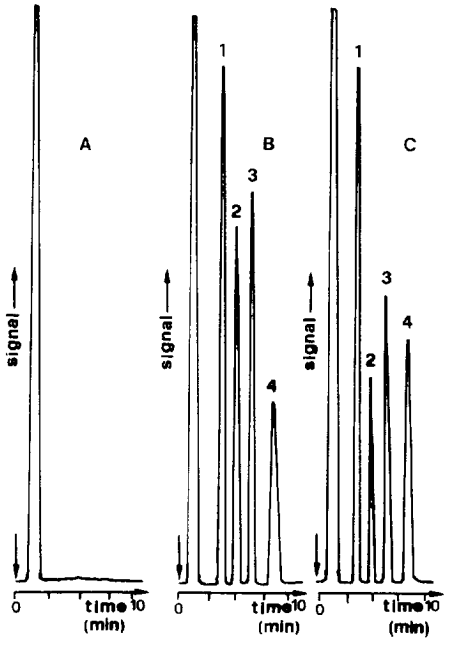

Fig. 2. HPLC profiles of human plasma extracts. (A) Drug-free human plasma; (B) drug-free human plasma spiked with 2.5 μg/ml rufloxacin (peak 4), 5.0 μg/ml fenbufen (peak 2), 3.0 μg/ml felbinac (peak 3) and 5.0 μg/ml internal standard (peak 1); (C) plasma sample from a volunteer (2 h after a dose of 400 mg of rufloxacin and 300 mg of fenbufen) containing 3.2 μg/ml of rufloxacin 4.1 μg/ml fenbufen and 1.8 μg/ml felbinac. Column, Zorbax SAX (250×4.6 mm I.D.); mobile phase, 0.1 *M* phosphate buffer (pH 7)–acetonitrile (90:10, v/v); UV detection at 280 nm.

The calibration graphs were linear from 0.2 to 10 μg/ml for rufloxacin, from 0.5 to 30 μg/ml for fenbufen and from 0.2 to 10 μg/ml for felbinac, respectively. The mean of seven different calibration graphs yielded the following equations: $y=1.48x-0.54$ for rufloxacin ($r=0.996$); $y=0.03x+1.15$ for fenbufen ($r=0.998$); $y=0.41x+0.02$ for felbinac ($r=0.997$), where y is the peak-area ratio between each drug and the internal standard in the arbitrary units of the HP-3396-II system used and x is the drug concentration (μg/ml).

The lower limits of detection, using a signal-to-noise ratio of three, were 0.1 μg/ml for rufloxacin, 0.3 μg/ml for fenbufen and 0.1 μg/ml for felbinac, respectively. The comparison of the peak-area ratios for rufloxacin, fenbufen and felbinac between fresh plasma samples and samples frozen at −20°C for a period of four weeks showed no differences. The HPLC method described in this paper allows the simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma. Since the inhibition of GABA receptor binding to synaptic membranes is dose dependent, it is useful to know the simultaneous concentrations of rufloxacin, fenbufen and felbinac. This method can be applied to pharmacodynamic studies of these compounds and will facilitate detailed investigations on the interactions between new quinolones and fenbufen.

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