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Short Communication

Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma

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ABSTRACT

An enantioselective high-performance liquid chromatographic assay for the quantitation of the enantiomers of flurbiprofen in human plasma is described. The procedure involved extraction of flurbiprofen from acidified plasma into hexane–diethyl ether (8:2, v/v). Stereoselective separation was achieved with a prepacked α_1-acid glycoprotein column without any derivatization procedure. A second assay using a conventional reversed-phase column to determine racemic flurbiprofen is also described. The detection wavelength was set at 246 nm. The limit of quantification was found to be 50 ng/ml for both assays. The method was demonstrated to be sufficiently sensitive for stereoselective pharmacokinetic studies of flurbiprofen.

INTRODUCTION

Flurbiprofen (FLU), a chiral 2-arylpropionic acid (2-APA) non-steroidal anti-inflammatory drug (NSAID), is used in its racemic form. The enantiomers of 2-APAs, however, may exert different pharmacodynamic and pharmacokinetic effects [1–5]. Thus, it is of scientific and clinical relevance to determine plasma concentrations stereoselectively.

Several methods have been reported for the detection of racemic FLU in plasma, *i.e.* thin-layer chromatography, gas chromatography and high-performance liquid chromatography (HPLC) [6–9]. Stereoselective assays for the determination of FLU in plasma, however, are rare. They are mainly based on the formation of diastereomeric derivatives [10–12], a rather time-consuming method that may be subject to a variety of errors [13]. We, therefore, developed a simple and rapid, but highly sensitive stereospecific HPLC assay for the determination of *R*- and *S*-FLU in plasma using a chiral α_1-acid glycoprotein (AGP) column [14] (assay I). A control of the stereoselective assay was enabled by the development of a racemic reversed-phase method (assay II), based

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on the same procedure of plasma sample preparation as the stereoselective assay.

EXPERIMENTAL

Chemicals

The enantiomers of FLU, 2-(2-fluoro-4-biphenylyl)propionic acid, were supplied by PAZ Arzneimittelentwicklungsgesellschaft (Frankfurt/Main, Germany). The optical purity of the *S*- and *R*-enantiomers was 98.5 and 99.1%, respectively. The internal standard (I.S.), *S*-naproxen (*S*-NAP), was purchased from Sigma (Deisenhofen, Germany). All other chemicals and organic solvents were of HPLC or reagent grade. The mobile phases were freshly prepared, filtered through 0.45-μm filters, and degassed under vacuum prior to use. The stock solutions were prepared by dissolving an appropriate amount of the FLU enantiomers in 0.03 *M* phosphate buffer (pH 7.5). Working standard solutions were prepared in drug-free plasma from the stock solution to yield concentrations from 25 to 20 000 ng/ml.

Stereoselective determination of flurbiprofen in human plasma (assay I)

The HPLC system consisted of a Model SP 8810 pump (Spectra Physics, Darmstadt, Germany), a Model SP 100 UV monitor (Spectra Physics) fitted with a Model 231 diluter autosampler (Gilson/Abimed, Langenfeld, Germany) and a CR 3A Shimadzu integrator (Shimadzu, Egling, Germany). Stereoselective separation was achieved with an AGP column (100 mm × 4.0 mm I.D., 5 μm, Grom, Herrenberg, Germany). Owing to the temperature dependence of the chiral separation [15,16], a column thermostat (Chemdata, Sinsheim, Germany) was used. The mobile phase consisted of 5% 2-propanol and 1 m*M* dimethyloctylamine (v/v) in 20 m*M* phosphate buffer (pH 6.5) at 15°C. The flow-rate was 0.8 ml/min. The detection wavelength was 246 nm. The system was used in an air-conditioned room (20°C).

Determination of total flurbiprofen in human plasma using a reversed-phase HPLC column (assay II)

The HPLC equipment used in assay II was similar in all respects to that employed in assay I. Separation was achieved with a prepacked column (100 mm × 4.0 mm I.D., Nucleosil 3 μm RP 8; Macherey and Nagel, Düren, Germany). The eluent was acetonitrile–distilled water (4:6, v/v) acidified with 1 ml of phosphoric acid (85%) per litre of eluent. The flow-rate of the mobile phase was 1 ml/min. The detection wavelength was 246 nm.

Analytical procedure

For the quantification of racemic FLU and its enantiomers, a 0.50-ml aliquot of human plasma was acidified by adding 0.20 ml of 2 *M* hydrochloric acid, followed by extraction into 6.00 ml of ice-cooled hexane–diethyl ether (8:2, v/v). After centrifugation (5 min at 1500 *g*), 5.00 ml of the organic layer were removed and evaporated to dryness under a gentle stream of dry nitrogen. The residue was redissolved in 0.50 ml of phosphate buffer (pH 7.5). Depending on the expected concentration the injection volume was 50 μl (high concentrations, 1–20 μg/ml) or 100 μl (low concentrations, 25–1000 ng/ml) for the stereoselective assay as well as for the racemic method. Standard curves were prepared by injecting extracted plasma samples spiked with various amounts of the *R*- and *S*-enantiomers of FLU, simulating the concentrations observed following administration of a single oral dose.

Precision of the assay

Standard curves were constructed by injecting plasma spiked with several concentrations of flurbiprofen enantiomers (assay I, 0.025–10 μg/ml; assay II, 0.050–25 μg/ml) Peak-area ratios were plotted *versus* concentrations of the standards.

Inter-day variability was determined with control samples that were extracted and injected daily (five-fold) on three successive days. Similarly,

within-day variability was assessed with five repeated injections, on the same day, for each of the nine (assay I) or seven (assay II) control samples. The flurbiprofen enantiomer concentrations were calculated from the standard curve run the same week.

The stability of the flurbiprofen enantiomers in plasma was assessed by analysing some quality control samples over the investigated concentration range after storing them frozen over a period of nine weeks. No degradation was detectable within this period of time.

Recovery values

Recovery values were determined by comparing extracted spiked samples (assay I, 0.025–10 μg/ml; assay II, 0.050–25 μg/ml) with unextracted standard solutions.

Application

The utility of the method was demonstrated after oral administration of 50 mg of *R*- and *S*-FLU to healthy volunteers. Blood samples were collected up to 24 h. The plasma was frozen immediately and stored at −30°C until analysis.

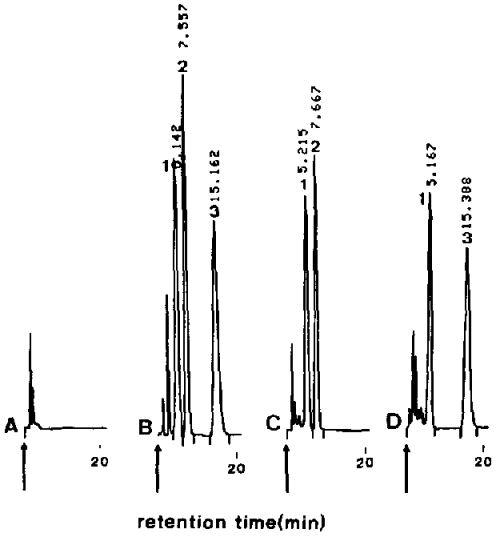

Fig. 1. Chromatograms of (A) an extracted blank human plasma sample, (B) plasma spiked with 5 μg/ml flurbiprofen racemate and 2.5 μg/ml internal standard *S*-naproxen, and human plasma samples 8 h following oral administration of (C) 50 mg of *R*-flurbiprofen and (D) *S*-flurbiprofen. Assay I. Peaks: 1 = *S*-naproxen; 2 = *R*-flurbiprofen; 3 = *S*-flurbiprofen.

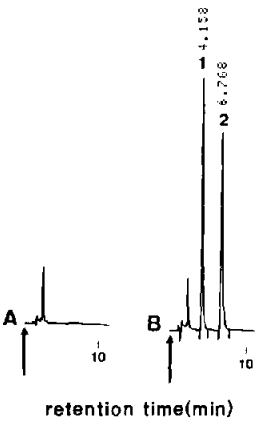

Fig. 2. Chromatograms of (A) an extracted blank human plasma sample and (B) plasma spiked with 5 μg/ml flurbiprofen racemate and 5 μg/ml internal standard *S*-naproxen. Assay II. Peaks: 1 = *S*-naproxen; 2 = flurbiprofen.

RESULTS AND DISCUSSION

Fig. 1 presents typical chromatograms of blank human plasma (A), plasma spiked with racemic FLU and *S*-NAP (I.S.) (B), and chromatograms of plasma samples obtained 8 h after oral administration of 50 mg of either *R*- or *S*-FLU to volunteers (C and D, respectively). Stereoselective chromatographic separation was completed within 15 min. The separation and chiral resolution factors of racemic FLU in plasma exceeded 1.6 and 2.0, respectively.

S-NAP proved to be a suitable I.S. for the stereoselective as well as for the racemic method (Fig. 2). The peak-area ratios of *R*- and *S*-FLU (assays I and II) to *S*-NAP were linearly related ($r > 0.999$) to the amount of the enantiomers added to blank human plasma in the range 25–10 000 ng/ml. The inter-day and intra-day precisions in plasma over three days and the recovery values are summarized in Tables I (assay I) and II (assay II). The quantification limit (less than 15% deviation of precision and/or accuracy values) was found to be 50 ng/ml for both the stereoselective and the racemic method. As the AGP column is based on immobilized protein, the separation power, which decreases with an increasing number of plasma samples, may also differ from one column to another. Thus, retention times, resolution and separation factors, and the quantification limit may vary to a limited extent depending

TABLE I

ANALYTICAL RECOVERY AND INTER- AND INTRA-DAY PRECISION OF *R*- AND *S*-FLURBIPROFEN OVER THREE DAYS (ASSAY I)

Concentration added	Recovery (n = 5) (%)	Concentration found (mean ± S.D., n = 5)			
		Day 1	Day 2	Day 3	Mean
R-FLU					
25 ng/ml	102.4 ± 5.2	29.7 ± 3.1	28.3 ± 2.2	28.6 ± 1.1	28.9 ± 0.7
50 ng/ml	101.0 ± 2.9	50.2 ± 2.9	48.3 ± 1.7	52.9 ± 3.9	50.5 ± 2.3
100 ng/ml	100.9 ± 2.7	98.7 ± 1.3	98.9 ± 3.5	92.9 ± 5.2	96.8 ± 3.4
250 ng/ml	99.4 ± 2.1	249.7 ± 4.8	249.1 ± 9.2	244.8 ± 7.5	247.9 ± 2.7
500 ng/ml	103.3 ± 2.4	492.2 ± 22.8	489.8 ± 9.8	501.1 ± 22.9	494.4 ± 5.9
1.0 μg/ml	96.5 ± 1.7	1.04 ± 0.05	0.98 ± 0.03	1.01 ± 0.02	1.01 ± 0.03
2.5 μg/ml	97.5 ± 0.7	2.51 ± 0.08	2.51 ± 0.07	2.52 ± 0.07	2.51 ± 0.01
5.0 μg/ml	98.3 ± 1.0	5.09 ± 0.06	5.00 ± 0.04	5.01 ± 0.09	5.03 ± 0.05
10.0 μg/ml	97.5 ± 1.0	10.00 ± 0.06	10.04 ± 0.09	10.00 ± 0.36	10.01 ± 0.02
S-FLU					
25 ng/ml	99.7 ± 5.6	22.5 ± 2.9	23.7 ± 4.1	24.8 ± 3.2	23.7 ± 1.2
50 ng/ml	97.2 ± 5.6	51.9 ± 3.5	51.8 ± 2.6	48.5 ± 3.1	50.7 ± 1.9
100 ng/ml	100.4 ± 3.8	102.4 ± 4.5	99.4 ± 4.1	99.1 ± 4.7	100.3 ± 1.8
250 ng/ml	98.2 ± 2.9	258.4 ± 7.2	242.2 ± 7.5	257.8 ± 8.5	252.8 ± 9.2
500 ng/ml	95.2 ± 5.2	492.8 ± 30.2	492.4 ± 15.7	496.1 ± 25.4	493.8 ± 2.0
1.0 μg/ml	95.6 ± 1.6	0.98 ± 0.04	0.94 ± 0.02	1.03 ± 0.05	0.98 ± 0.05
2.5 μg/ml	97.2 ± 2.1	2.57 ± 0.05	2.51 ± 0.20	2.53 ± 0.06	2.54 ± 0.03
5.0 μg/ml	98.0 ± 1.2	5.07 ± 0.07	5.08 ± 0.11	5.04 ± 0.05	5.06 ± 0.02
10.0 μg/ml	98.1 ± 1.0	9.98 ± 0.19	10.07 ± 0.13	9.99 ± 0.21	10.01 ± 0.05

on the column used. With the columns we have used, *ca.* 300–500 plasma samples per column could be normally reliably analysed.

The inter- and intra-day precision and the recovery values, however, prove that the method is sufficiently precise and sensitive for the quantification of FLU enantiomers in plasma.

Characteristic plasma concentration–time profiles for *R*- and *S*-FLU, following single oral administration of 50 mg of each enantiomer to vol-

TABLE II

ANALYTICAL RECOVERY AND INTER- AND INTRA-DAY PRECISION OF RACEMIC FLURBIPROFEN OVER THREE DAYS (ASSAY II)

Concentration added	Recovery (n = 5) (%)	Concentration found (mean ± S.D., n = 5)			
		Day 1	Day 2	Day 3	Mean
50 ng/ml	97.5 ± 12.8	57.1 ± 10.2	52.0 ± 6.9	60.9 ± 3.0	56.7 ± 4.5
100 ng/ml	103.2 ± 5.0	105.4 ± 4.8	101.4 ± 5.2	103.1 ± 4.2	103.3 ± 2.0
500 ng/ml	100.4 ± 3.0	519.3 ± 32.3	503.2 ± 1.9	491.4 ± 25.9	504.6 ± 14.0
1.0 μg/ml	98.8 ± 2.3	1.03 ± 0.04	1.02 ± 0.01	1.02 ± 0.02	1.02 ± 0.01
5.0 μg/ml	97.2 ± 1.1	5.20 ± 0.20	5.04 ± 0.27	5.11 ± 0.15	5.12 ± 0.08
10.0 μg/ml	100.6 ± 2.3	9.90 ± 0.06	9.98 ± 0.12	9.65 ± 0.87	9.84 ± 0.17
25.0 μg/ml	98.6 ± 1.1	25.17 ± 0.48	24.92 ± 1.09	25.10 ± 0.41	25.06 ± 0.13

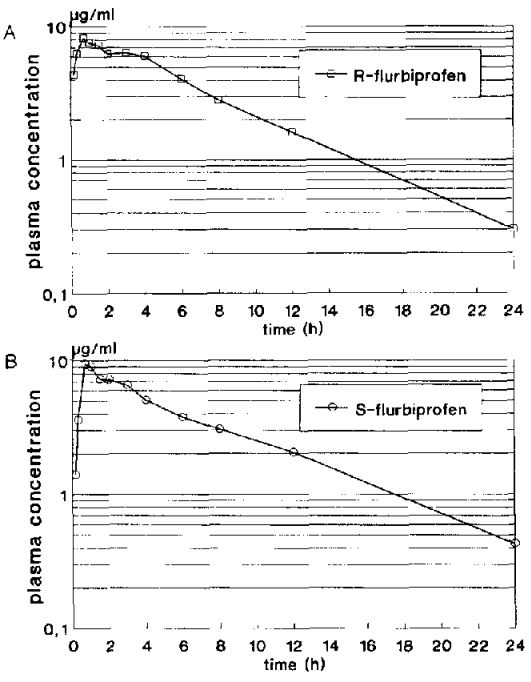

Fig. 3. Plasma concentration time profiles of (A) *R*-FLU following oral administration of *R*-FLU (50 mg) and (B) *S*-FLU following oral administration of *S*-FLU (50 mg), to healthy male subjects.

unteers, are shown in Fig. 3. No optical antipode could be detected in plasma after administration of either the *R*- or the *S*-enantiomer alone, *i.e.* there was no indication that inversion of *R*-FLU, which has been observed in several animal species [17], occurs in humans. These results are in line with findings published by Jamali *et al.* [4].

As the procedure for sample preparation is the same for the stereoselective and the non-stereoselective method, a rapid control of one assay by the other is possible at any time. A comparison of the plasma concentrations of the volunteers obtained by the stereoselective method and by the reversed-phase assay yielded good agreements, further indicating the lack of inversion of FLU in humans.

In conclusion, the described stereoselective HPLC method provides a simple, sensitive and reliable approach for the determination of the enantiomers of FLU in plasma after clinical doses, which is applicable for routine analysis.

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