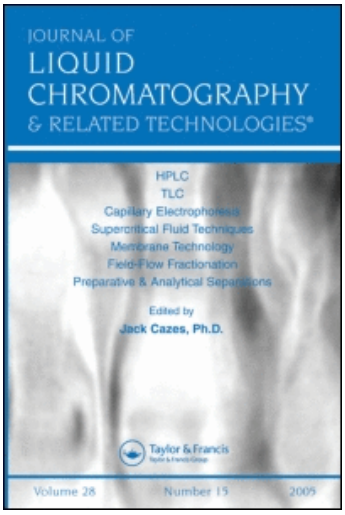

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SIMPLE CHIRAL LIQUID CHROMATOGRAPHIC SEPARATION OF FLURBIPROFEN ENANTIOMERS IN BIOLOGICAL FLUIDS

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ABSTRACT

An isocratic, simple liquid chromatographic method for the resolution of flurbiprofen enantiomers and their identification in urine samples was described. The resolution of flurbiprofen was achieved using cellulose tris (4-methylbenzoate) as a chiral stationary phases after derivatization to its corresponding methyl esters using diazomethane. The stereochemical resolution (R) obtained was 1.32 with baseline separation. The capacity factor (k) for the first eluted peak (-)-R-flurbiprofen was 1.99 and the stereochemical separation factor (α) obtained was 1.28. The method was applied to identify the flurbirprofen enantiomers in urine samples.

INTRODUCTION

Flurbiprofen chemically known as (±)-2-(2-fluoro-4-biphenyl) propionic acid is one of the nonsteroidal anti-inflammatory drugs (NSAID) which belongs to 2-arylpropionic acid class known as profens. These profens exist in two enantiomeric forms due to the presence of a chiral carbon atom alpha to the carbonyl function (Figure 1). It has been reported that the anti-inflammatory activity of flurbiprofen appears to be mainly due to the (+)-S-

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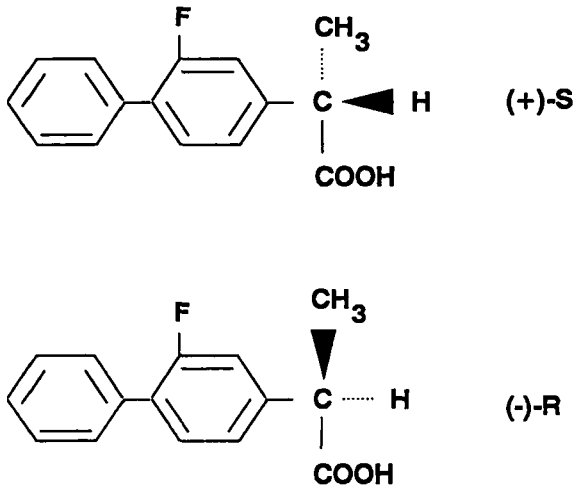

Figure 1. The absolute configuration of (-)-R- and (+)-S flurbiprofen enantiomers.

enantiomer[1]. Flurbiprofen, as the rest of 2-arylpropionic acids, also exhibit enantioselective pharmacokinetic aspects such as undirectional chiral inversion of the inactive (-)-R-enantiomer to the active (+)-S-enantiomer (2-6). However, in case of flurbiprofen this inversion is negligible[7].

Various high performance liquid chromatographic methods for the resolution of flurbiprofen enantiomers using chiral stationary phases (CSP's) has been reported. A series of 2-arylpropionic-acid NSAID was resolved on the (R)-N (3,5-dinitrobenzoyl)phenylglycine only after their conversion to amides (8). Furthermore, several direct resolution of underivatized profens have also been published. A variety of polysaccharide CSP's such as tris-(3,5-dimethyl-phenylcarbamate) of cellulose (Chiralcel OD) and amylose (Chiralpak AD) (9), and cellulose tris phenylcarbamate (Chiralcel OC) (10) and various protein CSP's such as bovine serum albumin (11), α_1-acid glycoprotein (12), ovomucoid (13) and human serum albumin (14, 15) were used in the enantiomeric separation of a number of profens including flurbiprofen.

Described here is an isocratic, simple method for the separation and identification of flurbiprofen enantiomers in biological fluids after derivatization to their corresponding methyl esters using diazomethane. The resolution was achieved on cellulose tris (4-methylbenzoate), namely, Chiralcel OJ column.

EXPERIMENTAL

Apparatus:

Waters LC system consisted of Model M-45 pump, a U6K injector, and a Lambda-max Model 481 LC Spectrophotometer UV detector set at 254 nm. The stationary phase of Chiralcel OJ analytical column of cellulose tris-(4-methyl benzoate) ester (25 cm x 4.6 mm, I.D., Daicel Chemical Industries, Tokyo, Japan) coated on silica gel with particle size 10μm were used.

Chemical:

Racemic flurbiprofen (Batch No. 810878), (+)-S-flurbiprofen, and (-)-R-flurbiprofen were kindly supplied by Boots Pharmaceuticals, Nottingham, England. HPLC grade 2-propanol, hexane and ethyl ether reagent grade were obtained from Fisher Scientific , Fairlawn, New Jersey, U.S.A. 1-Methyl-3-nitro-1-nitrosoguanidine was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A.

Chromatographic Conditions:

The maximum and symmetrical stereochemical resolution of flurbiprofen was obtained with hexane and 2-propanol (90:10) as eluent on chiralcel OJ column. Flow rate of 1.0ml/min and chart speed of 0.5cm/min. Temperature was maintained at 23°C and pressure at 300 psi. Detection was obtained at UV 254 nm with sensitivity range 0.01 AUFS. Sample amount injected was 3.0 nmole for racemic flurbiprofen and 1.5 nmole for

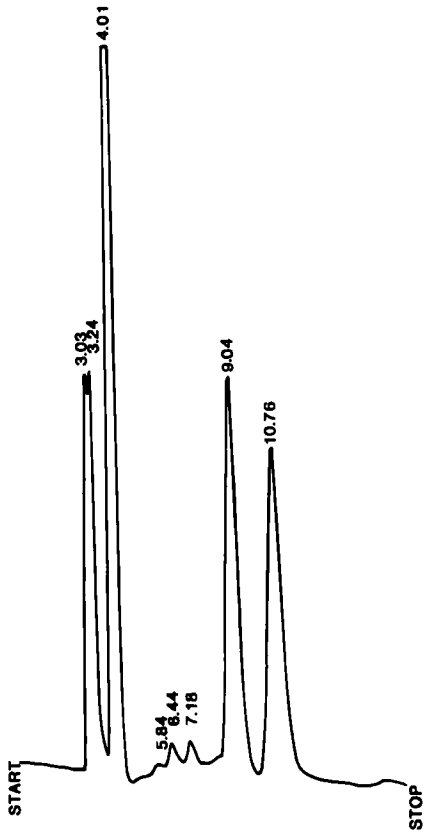

Figure 2. Enantiomeric separation of racemic flurbiprofen methyl esters. Column: Chiralcel OJ (250x4.6mm, i.d.); mobile phaseS: hexane/2-propanol (90:10); flow rate: 1.0 ml/min.; chart speed: 0.5 cm/min.; temperature: 23°C; detector: UV 254 nm; sensitivity: 0.01 AUFS; sample quantity: 3.0 nmole.

(+)-S-and (+)-R-enantiomers. These conditions also were used for the analysis of flurbiprofen in human urine sample.

Urine samples:

Human urine samples were collected for 12 hours after a healthy volunteer was administered 200 mg doses of flurbiprofen (Froben)®. Extraction of the drug with ether was performed, then the solvent was evaporated under stream of nitrogen.

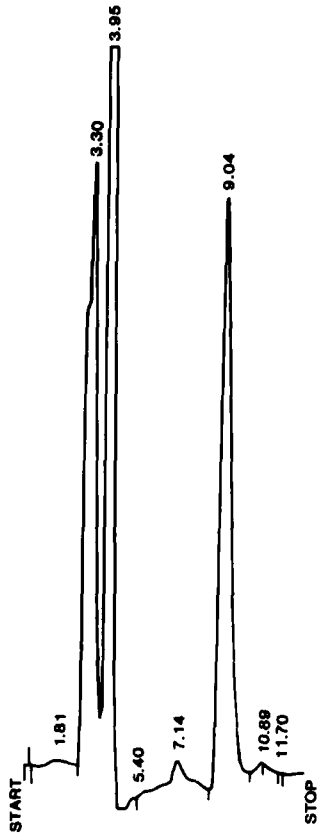

Figure 3. Chromatogram of (-)-R-flurbiprofen methyl ester. Conditions were the same as in Fig. 1, except the quantity of the sample injected was 1.5nmole.

Derivatization procedure:

Derivatization of flurbiprofen to the methyl ester was carried out using Aldrich (1-methyl-3-nitro-1-nitrosuguanidine) diazomethane apparatus (Aldrich Chemical Co., Milwaukee, WI, USA) which allows the preparation of diazomethane without distillation.

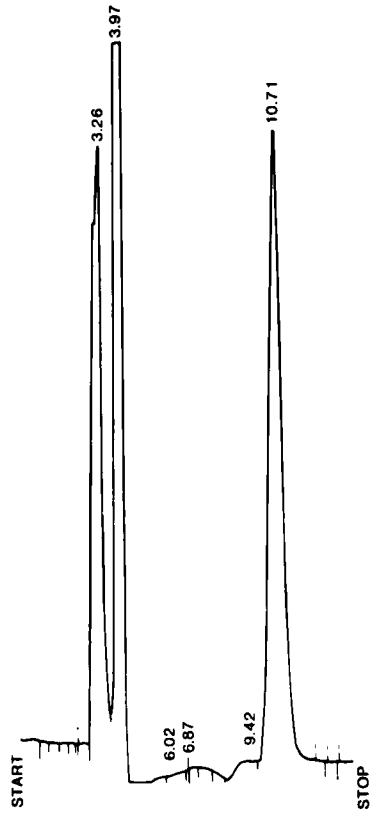

Figure 4. Chromatogram of (+)-S-flurbiprofen, methyl ester. Conditions were the same as in Fig. 1, except the quantity of the sample injected was 1.5nmole.

Determination of Enantiomeric Elution Order:

The enantiomeric elution order was determined by chromatographing the separate enantiomers under the same conditions. Thus the peak that eluted with a lower capacity factor was identified as (-)-R-flurbiprofen while the peak with higher capacity factor identified as (+)-S-flurbiprofen.

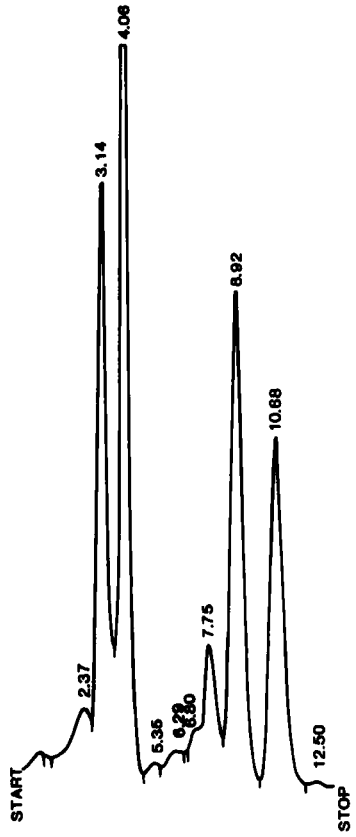

Figure 5. Chromatogram obtained from the ether extract of 100ml of urine sample collected from a healthy volunteer after administration of 200mg of racemic flurbiprofen and converted to their corresponding methyl esters. Chromatographic conditions were the same as in Fig. 1.

Results and Discussion

Direct resolution of flurbiprofen enantiomers on cellulose tris (4-methylbenzoate) ester (Chiralcel OJ) column was unsuccessful. The free acid did not elute through the chiral stationary phase. However, upon conversion of the free acid flurbiprofen to its methyl ester with diazomethane, chiral recognition was achieved and resolution of the

flurbiprofen enantiomers was successful. The method described here is simple and fast as it only requires about 45 minutes to run. It was found that profens in general were very strongly retained on protein CSP's and required 10% 1-propanol in 50 mM phosphate buffer at pH 7.6 and 1mM octanoic acid as a second modifier to reduce k and increase the α value which was reported to be 1.45 in case of flurbiprofen (11). A normal-phase eluent system is applied in this method which may be more convenient for preparative separation. Furthermore, the eluent does not contain second modifier e.g. octanoic acid as in case of protein CSP's or trifluoroacetic acid as in the case of Chiralpak AD column (9).

Different concentrations of 2-propanol in hexane were used as a mobile phase to optimize the separation of flurbiprofen. Typical chromatogram of the enatioseparation of flurbiprofen is shown in Figure 2. Compared with the chromatograms and capacity factors of (-)-R flurbiprofen (Figure 3) and (+)-S-flurbiprofen (Figure 4), the peak eluted at a lower capacity factor ($k_1 = 1.99$) was identified as (-)-R-enantiomer and the peak with the higher capacity factor ($k_2 = 2.55$) was identified as (+)-S-flurbiprofen. The stereochemical separation factor (α) was 1.28. The maximum stereochemical resolution factor (R) obtained was 1.32.

The enantiomeric separation of an ether extract of racemic flurbiprofen in urine samples collected from a healthy volunteer receiving 200 mg flurbiprofen tablets (Froben®) for 12 hours and after derivatization to their methyl esters is shown in Figure 5. It was observed from this determination that the undirectional chiral inversion of (-)-R enantiomer to the (+)-S-enantiomer is negligible. The ratio of the percent area of peaks representing (-)-R:(+)-S-flurbiprofen enantiomers was found to be 1.2:1 approximately. This data substantiate the results reported by Jamali et al (16) and also consistent with the findings of Knihinicki et al (17) which proves that the inactive (-) R-flurbiprofen enantiomer does not undergo undirectional bioinversion to the active (+) S-enantiomers.

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