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Short Communication

Simultaneous determination of (*R*)- and (*S*)-naproxen and (*R*)- and (*S*)-6-O-desmethylnaproxen by high-performance liquid chromatography on a Chiral-AGP column

Jan Vanggaard Andersen[☆] and Steen Honoré Hansen*

PharmaBiotec Research Centre, The Royal Danish School of Pharmacy, Department of Organic Chemistry, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)

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ABSTRACT

A high-performance liquid chromatographic method for the simultaneous determination of both enantiomers of naproxen and its metabolite 6-O-desmethylnaproxen has been developed. The separation is performed on a column containing α_1-acid glycoprotein as the chiral selector. The method has been used for the determination of the enantiomeric purity of the drug substance and the metabolite, and for the simultaneous determination of all four compounds in biological fluids.

INTRODUCTION

Naproxen, (*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, a non-steroidal anti-inflammatory drug of the 2-arylpropionic acid type, contains a stereogenic centre and is marketed as the pure (*S*)-(+)-enantiomer. However, commercially available samples of the drug and commercially available drugs containing (*S*)-(+)-naproxen also contain the (*R*)-(−)-enantiomer as an impurity. It is known that the activity of the drug resides almost exclusively in the (*S*)-(+)-enantiomer [1] and that the less active (*R*)-(−)-enantiomer is metabolically activated by inversion to the (*S*)-(+)-enantiomer, apparently a unidirectional pathway, which means that the (*R*)-(−)-enantiomer is a prodrug of the active (*S*)-(+)-enantiomer [2].

During a study of the metabolism of naproxen in humans [3], an analytical method for the determination of the enantiomeric purity of naproxen and 6-O-desmethylnaproxen (DM-naproxen) was needed.

Several methods, indirect and direct, have been reported for the separation of the enantiomers of naproxen and other 2-arylpropionic acids, but none of these include the separation of the enantiomers of the metabolite DM-naproxen. Indirect methods for the determination of the enantiomers of naproxen include derivatisation with L-leucineamide [4], *S*-1-phenylethylamine [5,6] and other optically active amines [7], fol-

[☆] Present address: Novo-Nordisk A/S, CNS Division, Department of Drug Metabolism, Novo Nordisk Park, DK-2760 Måløv, Denmark.

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lowed by separation in non-chiral high-performance liquid chromatographic (HPLC) or gas chromatographic systems. Most of the direct methods for the separation of the enantiomers of 2-arylpropionic acids are HPLC methods using chiral sorbents based on protein [*i.e.* α_1-acid glycoprotein (AGP)] adsorbed or immobilized on the silica surface [8–10]. AGP columns are becoming widely used in pharmaceutical analyses and the enantiomers of many different types of drugs are separated on the two types of column commercially available, Enantiopac or the second-generation column Chiral-AGP [10].

This paper describes a method for the simultaneous determination of (*R*)- and (*S*)-naproxen and (*R*)- and (*S*)-DM-naproxen in the drug substance and pure metabolite and in biological samples.

EXPERIMENTAL

Apparatus

A Kontron (Tegimenta, Switzerland) liquid chromatograph consisting of a Model 420 pump, a Model 460 autosampler, a Model 430 UV–visible detector operated at 232 nm, a Model 480 column oven and a Model 450 data system was used. A Jasco (Tokyo, Japan) 821-FP spectrofluorimeter operated at an excitation wavelength of 330 nm and an emission wavelength of 355 nm was also used.

Chemicals

(*R*)-Naproxen (99.7%) and (*S*)-naproxen (99.8%) were gifts from Syntex (Palo Alto, CA, USA). (*R*)-DM-naproxen (99.7%) and (*S*)-DM-naproxen (99.8%) were synthesized from (*R*)-naproxen and (*S*)-naproxen, respectively, as described previously [3]. β-Glucuronidase and sulphatase (*Helix pomatia*), 100 000 and 400–1000 U/ml, respectively, was from Sigma (St. Louis, MO, USA); N,N-dimethyloctylamine (DMOA) was from Aldrich (Steinheim, Germany). All other chemicals were of analytical-reagent grade from Merck (Darmstadt, Germany).

Animal experiments

Four female Sprague–Dawley rats weighing 190–200 g were used. Two rats were each given 2 mg of (*R*)-naproxen dissolved in 2 ml of 0.1 *M* potassium phosphate (pH 7.0) orally, and the other two rats were similarly given 2 mg of (*S*)-naproxen each orally. Urine was collected from each rat for 0–25 h.

Sample preparation

A 2.0-ml volume of rat urine was added to 2.0 ml of 0.2 *M* ammonium acetate (pH 5.0) containing 100 μl of β-glucuronidase. After 3 h at 37°C, 0.5 ml of glacial acetic acid was added and naproxen and DM-naproxen were extracted with 10 ml of diethyl ether–hexane (50:50, v/v). The aqueous phase was discarded and the organic phase was evaporated *in vacuo*. The residue was dissolved in the mobile phase and diluted to a final concentration of about 0.1 m*M*.

Standard samples of naproxen or DM-naproxen were dissolved in methanol and diluted with the mobile phase to a final concentration of about 0.1 m*M*.

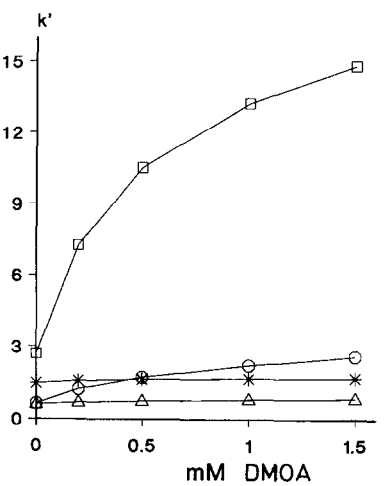

Fig. 1. Influence of the addition of DMOA to the mobile phase on the capacity factor (*k'*) of (*R*)- and (*S*)-naproxen and (*R*)- and (*S*)-DM-naproxen. Column, Chiral-AGP, 100 mm × 4 mm I.D.; eluent, 25 m*M* potassium phosphate (pH 7.0) containing 0.5% propan-2-ol and 0–1.5 m*M* DMOA; flow-rate, 0.7 ml/min; temperature, 25°C. Symbols: (△) (*R*)-DM-naproxen; (○) (*S*)-DM-naproxen; (✳) (*R*)-naproxen; and (□) (*S*)-naproxen.

Chromatography

The column used was a Chiral-AGP column (100 mm × 4 mm I.D.) from ChromTech (Norsborg, Sweden), operated at 25°C. The mobile phase was 25 m*M* potassium phosphate (pH 7.0) with 0.5% of an organic modifier (propan-2-ol) added. The mobile phase also contained 0–1.5 m*M* DMOA. The flow-rate was 0.7 ml/min and 20 μl of the final sample solution were injected onto the column.

RESULTS AND DISCUSSION

Hermansson and Eriksson [8,10] have shown that DMOA selectively changes the capacity factor (k') of the (*S*)-form of naproxen and other 2-arylpropionic acids. Using 25 m*M* potassium phosphate (pH 7.0) containing 0.5% propan-2-ol as mobile phase, it was found that (*S*)- and (*R*)-naproxen and (*R*)-DM-naproxen were well separated, whereas (*S*)-DM-naproxen had almost the same capacity factor as (*R*)-DM-naproxen. The addition of DMOA to the mobile phase selectively changed the capacity factor of the (*S*)-forms as expected. As indicated in Figs. 1 and 2, a satisfactory resolution of the four analytes was obtained by adding 1.5 m*M* DMOA to the eluent.

The extraction procedure has been described and validated previously [11]; a recovery of about

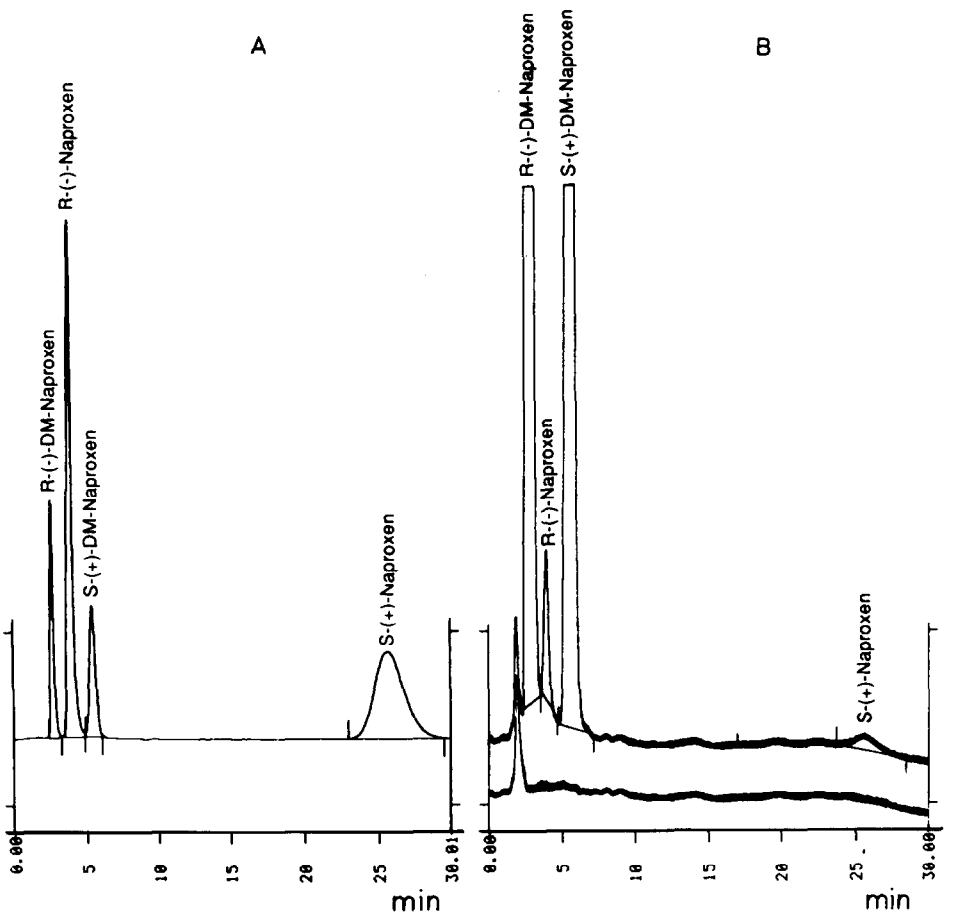

Fig. 2. (A) Simultaneous analyses of a racemate of DM-naproxen (50 μ*M*) and a racemate of naproxen (50 μ*M*). Injection volume: 20 μl. (B) Upper line: chromatogram of rat urine (0–25 h) obtained from a rat receiving 2 mg of (*R*)-naproxen orally. Lower line: blank urine from a rat. Concentration of solutes: (*R*)-naproxen, 1.9 μ*M*; (*R*)-DM-naproxen, 46.9 μ*M*; (*S*)-naproxen, 0.5 μ*M*; (*S*)-DM-naproxen (50.7 μ*M*). Injection volume: 20 μl. Chromatographic conditions as in Fig. 1, with 1.5 m*M* DMOA added to the mobile phase. Fluorescence detection at an excitation wavelength of 330 nm and emission at 355 nm.

100% and a good precision were demonstrated.

The method has been used for the determination of the optical purity of the naproxen and DM-naproxen standards used in this study (see under Experimental) and 0.1% of the (*R*)-enantiomer could be detected in the (*S*)-enantiomer, which is better than the performance of non-chromatographic or indirect chromatographic methods.

Two rats were treated with (*R*)-naproxen and the other two rats with (*S*)-naproxen to study the inversion of each enantiomer. It was found that 27 and 51% of the (*R*)-isomer was inverted to the (*S*)-isomer in the first two rats, respectively, whereas no inversion was observed when (*S*)-naproxen was administered. The minimum detectable amount of (*R*)-naproxen in urine from the rats was 0.01 nmol, corresponding to a detection limit of 5 pmol/ml of urine. In all four rats more than 95% of the dose was excreted in the urine as DM-naproxen or conjugates of DM-naproxen. In conclusion, even with this very sensitive method for the determination of the optical isomers, only the inversion of the (*R*)-form of naproxen derivative could be detected, which is a further indication of the unidirectional nature of the inversion.

REFERENCES

1 I. A. Harrison, B. Lewis, P. Nelson, W. Rooks, A. Roszkowski, A. Tomolonis and J. H. Fried, *J. Med. Chem.*, 13 (1970) 203.
2 J. M. Mayer, *Acta Pharm. Nord.*, 2 (1990) 197.
3 J. V. Andersen and S. H. Hansen, *J. Chromatogr.*, 577 (1992) 325.
4 R. T. Foster and F. Jamali, *J. Chromatogr.*, 416 (1987) 388.
5 J.-M. Maitre, G. Boss and B. Testa, *J. Chromatogr.*, 299 (1984) 397.
6 A. Rubin, M. P. Knadler, P. P. K. Ho, L. D. Bechtol and R. L. Wolen, *J. Pharm. Sci.*, 74 (1985) 82.
7 J. Bojarski, *J. Liq. Chromatogr.*, 12 (1989) 2585.
8 J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621.
9 G. Schill, I. W. Wainer and S. A. Barkan, *J. Chromatogr.*, 365 (1986) 73.
10 J. Hermansson, *Trends Anal. Chem.*, 8 (1989) 251.
11 J. W. A. van Loenhout, C. A. M. van Ginneken, H. C. J. Ketelaars, P. M. Kimenai, Y. Tan and F. W. J. Gribnau, *J. Liq. Chromatogr.*, 5 (1982) 549.