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Short Communication

Liquid chromatographic determination of the enantiomers of ibuprofen in plasma using a chiral AGP column

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method has been developed for the determination of the *R*- and *S*-enantiomers of ibuprofen. The enantiomers and the internal standard 4-pentylphenylacetic acid are extracted from plasma, separated and quantified on a Chiral-AGP column using ultraviolet detection. The simplicity, sensitivity and precision of the method makes it convenient for use in pharmacokinetic studies.

INTRODUCTION

Ibuprofen, *R,S*-2-(4-isobutylphenyl)propionic acid, is an important anti-inflammatory analgesic and antipyretic drug widely used in the treatment of rheumatic disorders, pain and fever [1]. The propionic acid side-chain contains an asymmetric α-carbon, giving two optical isomers. The drug is administered as the racemate, but only the *S*-form is responsible for the pharmacological effect. However, in humans, an inversion of the inactive *R*-form to the *S*-form occurs [2–5]. A number of methods for the chromatographic separation and quantification of ibuprofen enantiomers have been described. Separation after derivatization with an optically active reagent [5] or chiral stationary phases [5–9] has been used. Normally, the methods are based on two separate assays, one for the determination of the racemic drug and the other for the ratio of the *R*- and *S*-forms. This paper describes a single assay method for the quantitation of underivatized *R*- and *S*-ibuprofen in plasma samples.

EXPERIMENTAL

Chemicals

R,S-, *R*- and *S*-ibuprofen, and the internal standard 4-pentylphenylacetic acid

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(PPA) were kindly supplied by Boots (Nottingham, U.K.), N,N-Dimethyloctylamine was purchased from ICN Biomedicals (Plainview, NY, U.S.A.). Other chemicals were of HPLC or analytical grade.

Liquid chromatographic system

The solvent-delivery system was an LDC ConstaMetric III pump. Sample injections were performed with a Perkin-Elmer ISS-100 autosampler. The analytical column (100 mm × 4 mm I.D.) was a Chiral-AGP, α_1-acid glycoprotein immobilized on 5-μm sperical silica particles (ChromTech, Norsborg, Sweden). The UV absorbance was measured by a Spectra 100 (Spectra-Physics, San Jose, CA, U.S.A.). The chromatograms were recorded and analysed with P E Nelson Acces*Chrom GC/LC data system (Perkin Elmer Nelson Systems, Cupertino, CA, U.S.A.) where peak-area measurements were used.

Sample preparation

A 200-μl volume of PPA solution (100 μM) was added to each tube containing 0.25–1.0 ml of plasma, followed by 200 μl of 3 *M* hydrochloric acid and 6 ml of 0.1% (v/v) 2-propanol in *n*-hexane [4]. After gentle agitation in a rotating mixer for 15 min and centrifugation, the organic layer was transferred to a new tube and evaporated to dryness at 40°C under a stream of nitrogen.

The extraction residue was reconstituted in 0.2 ml of 0.01 *M* phosphate buffer (pH 7.4), vortex-mixed for 15 s and transferred to an injection vial. A 50-μl volume was injected into the chromatographic system. The plasma samples were stored in plastic tubes at −20°C until analysis.

Chromatography

The mobile phase consisted of 1.2% (v/v) 2-propanol and 1.2 m*M* N,N-dimethyloctylamine (DMOA) in 0.02 *M* sodium dihydrogenphosphate. The pH in the mobile phase was adjusted to 5.5 with 5 *M* sodium hydroxide. The flow-rate was 1.0 ml/min and the UV absorbance was monitored at 227 nm. Chromatography was performed at ambient temperature.

Quantification

Stock solutions of ibuprofen and the internal standard were prepared in phosphate buffer (0.01 *M*, pH 7.4) and stored at 4°C. Standard samples of ibuprofen in the range 1–60 μM were prepared by adding less than 1% (v/v) of the ibuprofen solutions to blank plasma. Quantifications were performed using a standard curve based on five duplicates of standard samples. The peak-area ratios from *R*- and *S*-ibuprofen relative to PPA were calculated, and a standard curve was constructed from the peak-area ratios and the standard concentrations.

RESULTS AND DISCUSSION

Regulation of retention

Variations of pH and the concentration of charged or uncharged modifiers influence the retention of and selectivity between *R*- and *S*-ibuprofen as demonstrated previously [9,10]. The retention and separation factor (α) could be regulated by small changes in pH and the concentration of 2-propanol. Addition of 1.2 m*M* DMOA improved the chiral separation and peak shape. Two Chiral-AGP columns from two batches were tested. The selectivity and retention data for ibuprofen and PPA are given in Table I. The differences between columns makes it necessary to optimize the mobile phase for each individual column. As shown in the table, mobile phase II could not be used with column I. A decrease in column efficiency was observed after 100–150 injections, but the column could be restored by pumping 25% (v/v) 2-propanol in water overnight at a low flow-rate.

Evaluation of analytical procedure

Fig. 1 shows typical chromatograms obtained from plasma samples processed according to the method. For the AGP column, normally less than 5 nmol is recommended as the injected amount. The calibration curve prepared from 1.0 ml of spiked plasma samples was found to be linear over the range 1–60 μM. Samples at concentration levels higher than 60 μM had to be diluted in order to retain column efficiency and enantioselectivity. The limit of quantification was set to 1 μM. At this level the inter-assay precision was *ca.* 10% (coefficient of variation, C.V.). Intra-assay precision calculated from ten spiked plasma samples, analysed on the same occasion, was investigated at three levels: 15, 30, and 60 μM. The C.V. was less than 10%.

TABLE I

INFLUENCE OF 2-PROPANOL AND pH ON SELECTIVITY AND RETENTION

Column I, Chiral-AGP Batch No. 89-26; column II, Chiral-AGP Batch No. 89-38. Mobile phase I, 1.2 m*M* DMOA and 1.2% (v/v) 2-propanol in 0.02 *M* sodium dihydrogenphosphate (pH 5.3); mobile phase II, 1.2 m*M* DMOA and 0.6% (v/v) 2-propanol in 0.02 *M* sodium dihydrogenphosphate (pH 5.7).

	Column I		Column II	
	Mobile phase I	Mobile phase II	Mobile phase I	Mobile phase II
k'_S	7.9	9.6	8.8	7.0
$\alpha_{S/R}$	1.3	1.5	1.3	1.2
$\alpha_{PPA/S}$	1.3	1.0	1.2	1.3

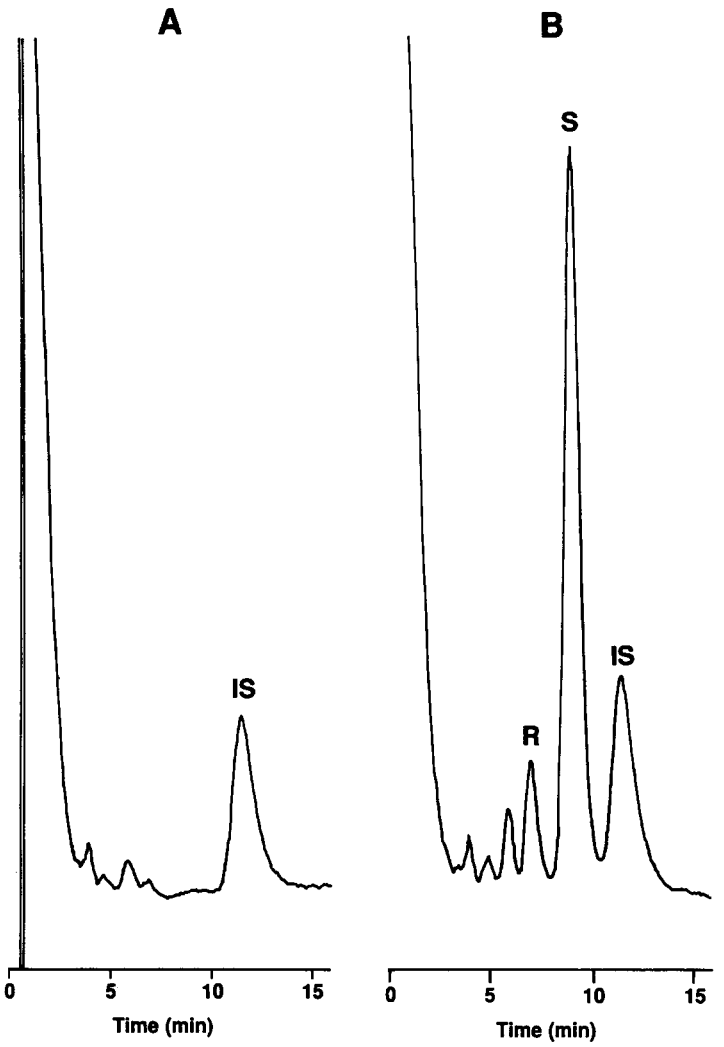

Fig. 1. Chromatogram from plasma obtained by analysis according to the described method. (A) Sample taken before dose. (B) Sample collected 7 h after a single oral dose of 800 mg of ibuprofen, containing 4 μM *R*-ibuprofen and 32 μM *S*-ibuprofen. Peaks: R = *R*-ibuprofen; S = *S*-ibuprofen; IS = internal standard, 20 μM 4-pentylphenylacetic acid.

CONCLUSION

The present method, using a single assay to determine *R*- and *S*-ibuprofen, is simpler than most of the methods reported in literature. The sensitivity and precision are satisfactory for pharmacokinetic work, and the method has been used to analyse samples from a pharmacokinetic study [11].

REFERENCES

1 S. S. Adams, K. F. McCullough and J. S. Nicholson, *Arch. Int. Pharmacodyn.*, 178 (1969) 115.
2 S. S. Adams, P. Bresloff and C. G. Mason, *J. Pharm. Pharmacol.*, 28 (1976) 256.
3 A. J. Hutt and J. Caldwell, *J. Pharm. Pharmacol.*, 35 (1983) 693.
4 E. J. D. Lee, K. M. Williams, G. G. Graham, R. O. Day and G. D. Champion, *J. Pharm. Sci.*, 73 (1984) 1542.
5 J. Bojarski, *J. Liq. Chromatogr.*, 12 (1989) 2685.
6 G. Li, G. Trieber and U. Klotz, *Drug Invest.*, 1 (1989) 11.
7 G. Geisslinger, K. Dietzel, D. Loew, O. Schuster, G. Rau, and G. Lachmann, *J. Chromatogr.*, 491 (1989) 139.
8 Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, *Chirality*, 1 (1989) 239.
9 M. Enqvist, *Doctorial Thesis*, Uppsala University, Uppsala, 1989.
10 G. Schill, I. W. Wainer and S. A. Barkan, *J. Chromatogr.*, 365 (1986) 73.
11 J. C. Nielsen, P. Bjerring, L. Arendt-Nielsen and K.-J. Pettersson, *Br. J. Clin. Pharm.*, 30 (1990) 711.