60P

STEREOSPECIFIC ASSAY OF IBUPROFEN AND ITS METABOLITES

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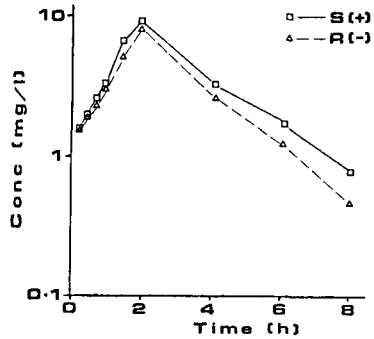
One of the features of the 2-arylpropionic acid class of anti-inflammatory drugs is the presence of a centre of chirality leading to two enantiomeric forms. When administered as a racemate, interconversion occurs from the pharmacologically inactive R(-) enantiomer to the active (S+) enantiomer (Hutt & Caldwell 1983). Therefore to study the pharmacokinetics of these drugs, sensitive, stereoselective assays are required for the separation of the enantiomers of the drugs and their metabolites. Previous analytical techniques have employed HPLC (Maitre et al 1984) and, whilst sensitive enough for pharmacokinetic studies, lack the sensitivity for measuring the unbound concentration of these drugs which are highly bound to plasma proteins. We have developed gas chromatography-mass spectrometric (GC-MS) assays for the enantiomers of ibuprofen in plasma (bound and unbound) and its metabolites in urine and applied these assays to a preliminary pharmacokinetic study.

Samples were extracted from plasma or urine, derivatised with S(-)phenylethylamine in the presence of 1,1'-carbonyldiimidazole to form, via a 1-arylimidazole intermediate, diastereoisomeric amides. After further work-up and extraction the samples were injected onto the GC-MS. 2-chlorobiphenylpropionic acid was used as the internal standard. The separations were carried out on a DB-5 capillary column (30m x 0.25mm), with ibuprofen derivatives eluting at 12.9 and 13.2 minutes at 250°C, after which the temperature was increased to 290°C in 1 min and the internal standard derivatives eluted at 17.0 and 17.5 minutes. The fragmentation pattern of the derivatives was characterised and the ions at m/z 161 for ibuprofen (C-alkyl cleavage) and m/z 363 for the internal standard (molecular ion) were chosen for quantitative purposes using selective ion monitoring (SIM). The limit of detection for both diastereoisomers was approximately 10µg/l.

In man, the two major metabolites of ibuprofen are formed by ω-2-hydroxylation (hydroxy metabolite) and ω-1-hydroxylation followed by oxidation (carboxy metabolite), the latter producing a second chiral centre in the molecule. Both the metabolites were derivatised as for the parent drug and analysed by GC-MS. The diastereoisomers of the hydroxy metabolite had retention times of 8.7 and 8.9 minutes at 300°C. The four chiral species of the carboxy metabolites (SS, RS, SR, RR) only produced three peaks, eluting at 23.6, 25.4 and 26.8 minutes at 300°C. The second peak (25.4 min) was never fully resolved and had twice the response of the other two species. The derivatives of both metabolites showed no molecular ion but side chain fragmentation of the hydroxy metabolite derivative produced an ion at m/z 267 and C-alkyl cleavage with rearrangement of the carboxy metabolite derivative produced an ion at m/z 295, both ions being suitable for SIM. The limit of detection for all species was approximately 10µg/l.



The analytical methodology was applied to a preliminary study of the pharmacokinetics of the enantiomers of ibuprofen in man. The concentration-time profiles of the enantiomers following oral administration of 400mg racemic ibuprofen to a normal volunteer are shown in the figure. The AUC (0,∞) for S(+) ibuprofen (29.9 mg/l x h) was larger than that for R(-) ibuprofen (25.7 mg/l x h) and the half-life of S(+) enantiomer (1.7h) was slightly longer than that of the R(-) enantiomer (1.6h).

Hutt, A.J., Caldwell, J. (1983) J. Pharm. Pharmacol. 35: 693-704
Maitre, J.M. et al (1984) J. Chromatogr. 299: 397-403