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DETERMINATION OF THE ENANTIOMERIC COMPOSITION OF IBUPROFEN IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A normal-phase high-performance liquid chromatographic method, using a hexane–ethyl acetate solvent system, for the determination of the enantiomeric composition of ibuprofen in human plasma is described. The method is based on the resolution of the diastereoisomeric amides formed on reaction of the ibuprofen enantiomers with *S*-1-(naphthen-1-yl)ethylamine using *p*-chlorophenoxyacetic acid as internal standard. The application of the method for the determination of the enantiomeric composition of ibuprofen in human plasma following the repeated oral administration of the drug to two volunteers is reported. The plasma concentrations of the *S*-(+) enantiomer were always greater than that of the *R*-(−), the ratio of the areas under the enantiomer plasma concentration–time curves (*S*/*R*) being 1.8 and 1.6.

INTRODUCTION

There has been considerable interest recently in the action, pharmacokinetics, metabolism and clinical pharmacology of the enantiomers of chiral drug molecules [1–5]. This interest has been stimulated in part by the development of chromatographic methods suitable for both the preparative resolution and analytical determination of enantiomers in biological fluids [6,7].

Of particular importance is the stereochemistry of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs (NSAIDs), the profens, which possess a chiral centre in the propionic acid moiety. With the exception of *S*-(+)-naproxen these compounds are marketed as racemic mixtures; however, they are stereospecific in action [4,8] and also undergo metabolic chiral inversion from the inactive *R*-(−) enantiomers to their pharmacologically active *S*-(+) antipodes [9–12]. The extent of the inversion reaction depends on the structure of the acid in question and the species of animal under investigation [9]. Thus the determination

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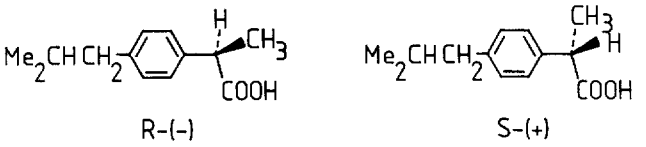


Fig. 1. Stereoisomers of ibuprofen, 2-(4-isobutylphenyl)propionic acid.

of the enantiomeric composition of these compounds in biological fluids is extremely important in both pharmacological and toxicological studies, so that either human or animal exposure to the active agent may be accurately determined.

Several general methods for the high-performance liquid chromatographic (HPLC) resolution of the enantiomers of the profens have been reported, including the resolution of the diastereoisomeric amides formed on reaction with *S*-(−)-1-phenylethylamine, using 1,1′-carbonyldiimidazole as a coupling agent, followed by normal-phase chromatography using a stainless-steel column [13]; resolution of the diastereoisomeric amides formed on reaction of *S*-1-(naphthen-1-yl)ethylamine, using a carbodiimide as the coupling agent, followed by normal-phase chromatography using a radial compression column [14]; resolution of diastereomeric ion pairs using quinine as a chiral counter ion [15]; the use of chiral stationary phases, e.g. (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, after derivatisation of the acids to their corresponding 1-naphthalenemethylamides [16] or benzylamides [17], and α_1-acid glycoprotein (α_1-AGP) phases [18].

Of this series of NSAIDs, ibuprofen [*R,S*-2-(4-isobutylphenyl)propionic acid, Fig. 1] was the first compound demonstrated to undergo stereoselective metabolic chiral inversion [9–11,19] and many of the reports cited above include ibuprofen, together with related 2-arylpropionic acid NSAIDs, to demonstrate the application of the chosen method of resolution [13,14,16–18]. However, there are only two published methods for the quantitative analysis of the enantiomers of ibuprofen in human plasma [10,20,21]. The first involves the formation of the *S*-α-methylbenzylamides of the enantiomers of ibuprofen, using 1,1′-carbonyldiimidazole as a coupling agent, followed by the gas chromatographic resolution of the diastereoisomeric amides [20]. This method has also been extended to determine the enantiomeric composition of the two major metabolic oxidation products of the drug [10]. The above method involves a tedious thin-layer chromatographic work-up step, after the extraction of the drug-related material from plasma, and it has been reported that the quantities of the coupling agent used were less than optimal for efficient derivatisation [13]. A more recent method [21] involves the derivatisation of the enantiomers using acidified *S*-2-octanol followed by HPLC resolution of the diastereoisomeric esters formed.

The present paper describes an HPLC method for the determination of the enantiomeric composition of ibuprofen in human plasma based on our previously reported derivatisation technique for the formation diastereoisomeric *S*-1-(naphthen-1-yl)ethylamides [14].

EXPERIMENTAL

Compounds

The following compounds were purchased: *S*-(−)-1-(naphthen-1-yl)ethylamine, 1-hydroxybenzotriazole (1-HOBT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and *p*-chlorophenoxyacetic acid from Aldrich (Gillingham, U.K.); *R,S*-, *R*-(−)- and *S*-(+)-ibuprofen were the generous gifts of Dr. V.R. Holland, Boots (Nottingham, U.K.). Solvents for HPLC were purchased from Koch-Light (Haverhill, U.K.); all other reagents were of analytical grade or better and were used without further purification.

Instrumentation

Chromatography was carried out using a Perkin-Elmer Series 10 liquid chromatograph linked to an LC-75 UV detector, set at 254 nm, and a Perkin-Elmer R-100 chart recorder and equipped with a Hypersil column (250×4.5 mm I.D., 10 μm particle size). The mobile phase was hexane–ethyl acetate (4:1) at a flow-rate of 3.2 ml min^{-1} at ambient temperature. Samples were introduced into the system via a Rheodyne valve loop injector (20-μl loop).

Sample preparation

Standard solutions of mixtures of *R*-(−)- and *S*-(+)-ibuprofen, containing 0.5–2.5 and 2.5–25 μg of the individual enantiomers, were prepared by mixing appropriate volumes of stock solutions of the enantiomers in dichloromethane, to yield final total concentrations of 2.5 and 25 μg ml^{-1} ibuprofen in plasma. To the above solutions were added 100 μl of the internal standard solution containing *p*-chlorophenoxyacetic acid (60 μg ml^{-1}). The solutions were mixed and evaporated to dryness using a rotary evaporator. The residue was taken up in heparinized drug-free plasma (0.5 ml) and to this was added 1.28 *M* hydrochloric acid (0.2 ml) and the whole extracted with benzene (5 ml). The samples were vortex-mixed for 5 min and the phases separated by centrifugation (400 *g* for 15 min). The organic phase was removed and evaporated to dryness using a rotary evaporator and the residues taken up in dichloromethane (1 ml) and derivatised as described below. Analysis of plasma samples was carried out by the addition of 100 μl of internal standard solution (*p*-chlorophenoxyacetic acid, 60 μg ml^{-1}) and hydrochloric acid (0.2 ml) to 0.5 ml of heparinized plasma, followed by 5 ml of the extraction solvent (benzene) and the mixture treated as described above. The extraction efficiency was estimated by carrying out the derivatisation procedure omitting the initial evaporation and extraction steps.

The derivatisation procedure employed was similar to our previously reported method [14]. The extracted ibuprofen enantiomers and the internal standard in dichloromethane were derivatised by the addition of 1-hydroxybenzotriazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and *S*-1-(naphthen-1-yl)ethylamine (100 μg of each as 100 μl of 1 mg ml^{-1} solutions in dichloromethane). The solutions were mixed and allowed to stand at room temperature for 2 h; after this time the solvent was evaporated off and the residue

taken up in HPLC solvent (0.5 ml) and 20-μl aliquots were injected onto the column.

Standard curves were prepared by comparison of the peak-height ratios of each ibuprofen amide derivative to the internal standard amide derivative versus the concentration of the ibuprofen enantiomer.

Human study

Two healthy volunteers were each given three separate doses, at 6-h intervals, of ibuprofen as 200-mg tablets, the first dose following a light breakfast. Blood samples (10 ml), obtained by venepuncture, were collected into heparinised tubes at the following times 0, 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 16 and 24 h. The plasma was separated immediately by centrifugation and was stored at $-20°$C until analysed as described above.

RESULTS AND DISCUSSION

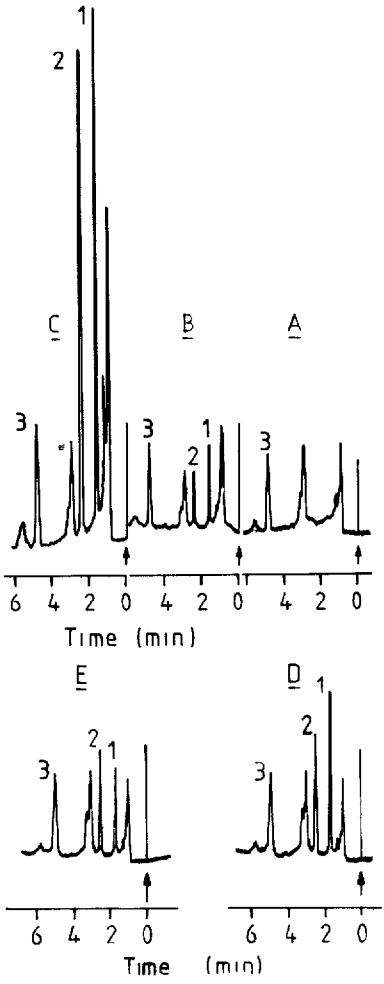
Chromatography

The *R,S* and *S,S* diastereoisomeric amides of ibuprofen and the amide of the internal standard, *p*-chlorophenoxyacetic acid, were well resolved with retention times of 1.6, 2.5 and 4.9 min, respectively. Typical chromatograms obtained using the technique described above are shown in Fig. 2. The total run time for each sample was 6 min, which was considerably shorter than the 30 min when using the previously reported HPLC method for the analysis of ibuprofen enantiomers [21]. An additional advantage of the present method is the increased stability of the diastereoisomeric amide derivatives over that of esters, which has been proposed to account for the limited use of chiral alcohols, compared to chiral amines, as resolving agents in bioanalytical work [7]. Using the isolation procedure described no interfering peaks due to the normal constituents of human plasma were observed in the chromatograms and the extraction efficiency using benzene was found to be $80 \pm 1.4\%$.

The elution order of the diastereoisomeric *S*-1-(naphthen-1-yl)ethylamides of ibuprofen was *R,S* followed by *S,S*, in agreement with previously reported data [13,14], the separation (α) and resolution (R) factors being 2.29 and 3.60, respectively. The separation factor (α) was calculated from $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the capacity ratios for the first- and second-eluting peaks determined by reference to an unretained compound, and the resolution factor (R) from $2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times of the first- and second-eluting peaks and w_1 and w_2 are the widths of the peaks at their bases.

Derivatisation

The derivatisation procedure used was essentially that described in our previous report [14] with only slight modifications in the concentration of the reagents used, and an increase in reaction time, to optimise the conditions for ibuprofen. As previously reported the technique is essentially quantitative and does not cause stereoselective derivatisation of the enantiomers [14]. A similar method, using dicyclohexylcarbodiimide, has been described recently for the deri-



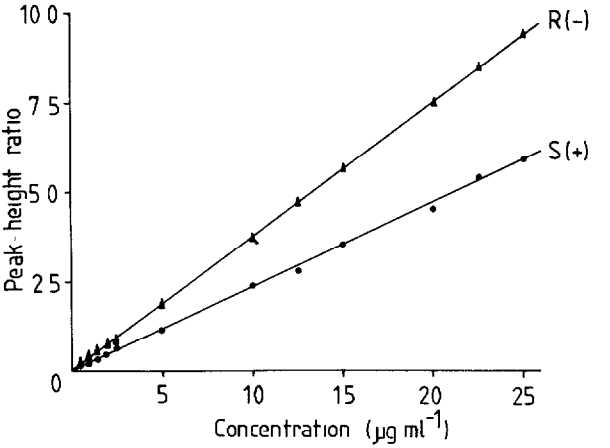


Fig. 2. Typical chromatograms obtained from: (A) drug-free plasma; (B) spiked plasma containing 2.5 μg ml^{-1} *R*-(−)- and 3.0 μg ml^{-1} *S*-(+)-ibuprofen; (C) spiked plasma containing 11.0 μg ml^{-1} *R*-(−)- and 17 μg ml^{-1} *S*-(+) ibuprofen; and (D, E) plasma samples at 1 h (D) and 4 h (E) after the oral administration of 200 mg ibuprofen to a volunteer. Peaks: *S*-1-(naphthen-1-yl)ethylamides of (1) *R*-(−)-ibuprofen, (2) *S*-(+)-ibuprofen and (3) internal standard, *p*-chlorophenoxyacetic acid.

Fig. 3. Calibration curves of the *R*-(−) and *S*-(+) enantiomers of ibuprofen after derivatisation with *S*-1-(naphthen-1-yl)ethylamine.

vatisation of the enantiomers of loxoprofen and its metabolites [22].

Calibration curves

Standard curves were constructed over the range 0.5–25 μg ml^{-1} for each enantiomer, as mixtures, containing either 2.5 or 25 μg ml^{-1} total ibuprofen, by comparison of the peak heights of each diastereoisomeric amide of ibuprofen with those of the amide formed by derivatisation of the internal standard (Fig. 3). The curves were linear over the range examined, yielding correlation coefficients

TABLE I

REPRODUCIBILITY OF THE STANDARD CURVES

Two ranges of total ibuprofen concentration were used for the preparation of the standard curves: 2.5 and 25.0 μg ml^{-1}. The data summarised were collected over several weeks ($n=5$).

Enantiomer concentration (μg ml^{-1})		Mean peak-height ratio		Coefficient of variation (%)	
R-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)
-	25.0	-	5.95	-	1.7
2.5	22 5	0.95	5.38	3.5	0.4
5.0	20.0	1.91	4.55	1.7	1 8
10 0	15 0	3.81	3.62	2.0	2.3
12.5	12.5	4.77	2.86	2.2	2.0
15 0	10 0	5.67	2.38	2.1	1.1
20 0	5 0	7.59	1.14	0.8	4.8
22.5	2.5	8.57	0.62	3 0	4.5
25.0	-	9.50	-	2.1	-
0.5	2.0	0.19	0.48	4 1	3.3
1 0	1 5	0.38	0.36	3.1	2.1
1.5	1.0	0.57	0 23	4 1	3.7
2.0	0 5	0.76	0 12	2.9	1.8

of 0.999, almost passing through the origin with intercept values ca. 3% of the lowest peak-height ratio determined. The reproducibility of the calibration curves is presented in Table I and the inter-sample variability in Table II.

The sum of the individual enantiomers of ibuprofen (determined as described above) were compared with results obtained for racemic ibuprofen using a direct-injection technique [23]. All analyses were performed using human plasma samples ($n=55$) and a linear relationship (correlation $r^2>0.99$) was obtained, values of slope and intercept being 1.003 and 0.0042, respectively.

Application

Plasma samples from two healthy volunteers following the repeated 6-h oral administration of 200-mg ibuprofen tablets were analysed using the method described above. Plasma concentration–time curves for the individual enantiomers and total ibuprofen are shown in Fig. 4. The plasma concentrations of the *S*-(+) enantiomer were found to be greater than that of its *R*-(−) antipode in

TABLE II

INTER-SAMPLE REPRODUCIBILITY OF THE ANALYTICAL METHOD

All samples were analysed on the same day; $n=10$.

Total ibuprofen concentration (μg ml^{-1})	Enantiomer	Enantiomer concentration (μg ml^{-1})		Coefficient of variation (%)
		Theoretical	Measured (mean ± S.D.)	
2.5	*R*-(−)	1.0	1.01 ± 0.014	1 4
	S-(+)	1.5	1.54 ± 0.025	1.6
25.0	*R*-(−)	10.0	10.25 ± 0.12	1 1
	S-(+)	15.0	15.51 ± 0.20	1.3

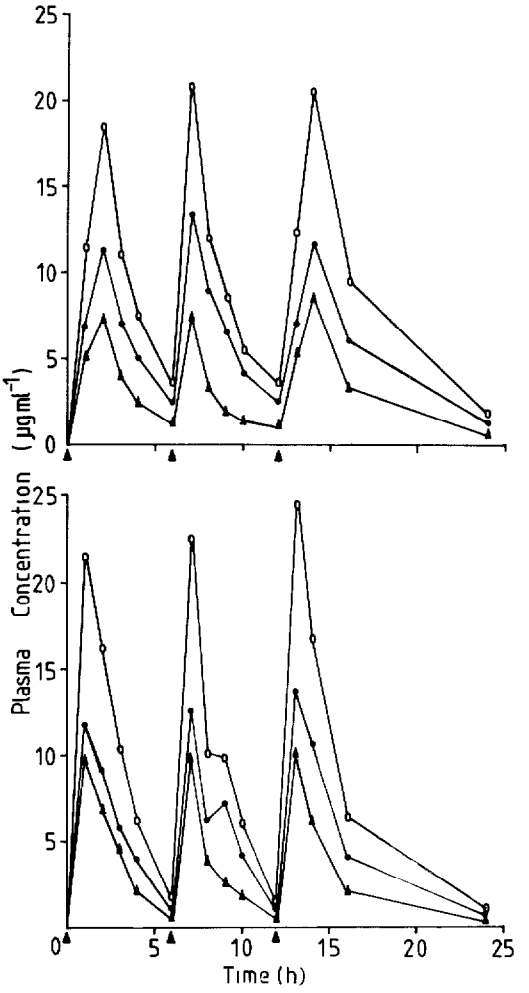


Fig. 4. Plasma levels of *R*-(−) (▲), *S*-(+) (●) and total (○) ibuprofen in samples from two volunteers after the oral administration of 200-mg ibuprofen tablets every 6 h (arrow heads indicate time of drug administration).

all samples examined, in agreement with previous reports [12,21]. Areas under the plasma concentration–time curve (AUC) were calculated using the trapezoidal rule and the values of peak plasma concentration, time to reach peak plasma concentration and the elimination half-life, estimated by least-squares regression analysis, are presented in Table III. The proportion of the total drug AUC due to the *S*-(+) enantiomer, i.e. exposure of the volunteers to the active agent, was found to be ca. 63% in agreement with results previously reported by Lee et al. [12] who examined the pharmacokinetics of ibuprofen enantiomers after single oral dose administration.

In conclusion, the analytical method reported herein enables the determination of the enantiomeric composition of ibuprofen in human plasma, with a shorter chromatographic run time than that previously described [21] and at a sensitiv-

TABLE III

PHARMACOKINETIC PARAMETERS OF THE ENANTIOMERS OF IBUPROFEN IN TWO HEALTHY VOLUNTEERS AFTER REPEATED 6-h ADMINISTRATION OF 200 mg RACEMIC IBUPROFEN IN A TABLET FORMULATION

Parameter	Subject 1		Subject 2	
	S-(+)	*R*-(−)	*S*-(+)	*R*-(−)
Highest observed plasma concentration ($\mu g\ ml^{-1}$)★	11.3	7.3	11.8	9.8
Time to highest observed plasma concentration (h)★	2	2	1	1
Elimination half-life (h)	1.9	1.6	1.9	1.2
Area under plasma curve 0–24 h ($\mu g\ h\ ml^{-1}$)	135.4	75.9	122.3	76.6
Proportional AUC (%)	64.1	35.9	61.5	38.5

★Estimated from the first dose.

ity which is suitable for the determination of the plasma concentrations of total drug found in biopharmaceutical and therapeutic studies i.e. 1–66 $\mu g\ ml^{-1}$ [24,25].

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