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Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1-acid glycoprotein column

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ABSTRACT

The effect of mobile phase composition, pH and temperature on the chiral resolution and retention of some 2-arylpropionic acids using the chiral α_1-acid glycoprotein column EnantioPac® is described. Furthermore, a direct stereoselective high-performance liquid chromatographic assay to determine the enantiomers of ketoprofen, ibuprofen and fenoprofen in plasma is presented. Detection was at 260, 220 and 220 nm for ketoprofen, ibuprofen and fenoprofen, respectively. The limit of detection was 0.1 μg/ml for the enantiomers of ketoprofen and ibuprofen, and 0.25 μg/ml for the enantiomers of fenoprofen. The method was demonstrated to be applicable for stereoselective pharmacokinetic studies of ketoprofen, ibuprofen and fenoprofen after administration under clinical conditions.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) including 2-arylpropionic acids (APAs) such as ketoprofen (KT), ibuprofen (IBU) and fenoprofen (FENO), are in clinical use in their racemic forms. It is well known that the enantiomers may exert different pharmacodynamic and pharmacokinetic effects [1–4]. Consequently, the enantioselective investigation of pharmacokinetic parameters is of scientific and medical relevance.

Three methods are currently used to achieve chromatographic enantioselective resolution of racemic compounds: (i) formation of diastereomeric derivatives prior to HPLC separation [5–7]; (ii) addition of chiral complexing agents to the mobile phase [8,9]; and (iii) the use of chemically bonded chiral stationary phases (CSPs) [8]. A considerable number of CSPs are today commercially available, including ligand-exchange phases, affinity phases, helical polymer phases and chiral cavity-type phases [10–12].

The enantioselective separation of basic, acidic and non-protolytic compounds by means of the chiral α_1-acid glycoprotein (AGP) column EnantioPac was published recently [13–21]. In these investigations particular attention was paid to the extension of the range of racemic compounds separable by an AGP column.

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Furthermore, eluent effects on the enantioselective resolution were analysed to achieve optimized enantiomeric separation.

The major intention behind our experiments, however, was to examine the applicability of the EnantioPac column for the quantification of the enantiomers of some APAs in plasma. As no general requirements of the mobile phase composition for the separation and retention of racemic compounds are known, the effects of the mobile phase composition, pH and temperature were investigated to define acceptable separation conditions for the determination of KT, IBU and FENO in plasma samples

EXPERIMENTAL

Chemicals

The KT and IBU enantiomers were supplied by Rhône Poulenc (Paris, France) and Pharma Trans Sanaq (Basel, Switzerland), respectively. The racemic compounds of ketoprofen, ibuprofen, fenoprofen, indoprofen, carprofen and tiaprofenic acid were purchased from Sigma Chemie (Deisenhofen, F.R.G)

All other chemicals and organic solvents were of HPLC or reagent grade. The mobile phases were freshly prepared, filtered (0.45 μm) and degassed under vacuum prior to use. The stock solutions of the substances were prepared by dissolving an appropriate amount of the compounds in 0.03 *M* phosphate buffer (pH 7.5). Working standard solutions of KT, IBU and FENO were prepared in drug-free plasma from the stock solution to yield the required concentration ranges.

Apparatus

The HPLC equipment consisted of a System Gold Module 126 pump, a System Gold Module 166 programmable detector (Beckman, Frankfurt/Main, F.R.G.), a Gilson 231 dilutor (401) autosampler (Abimed, Langenfeld, F.R G.) and a CR3A Shimadzu integrator (Egling, F.R.G.). Detection was at 260, 220 and 220 nm for KT, IBU and FENO, respectively. The injection volume was 40 μl.

For stereoselective separation an AGP column (EnantioPac, LKB/Pharmacia, Freiburg, F.R.G., 100 mm × 4.0 mm I.D.) was used. Owing to the temperature dependence of the chiral separation, a column thermostat (Chemdata, Sinsheim, F.R.G.) was utilized.

Assay I: variation of mobile phase composition and temperature

The effect of pH, 2-propanol concentration and the content of dimethyloctylamine (DMOA) in the mobile phase, as well as of temperature, on the separation and capacity factors was analysed by varying the parameters of the following starting chromatographic conditions: 0.5% 2-propanol in 0.02 *M* aqueous phosphate buffer with 5 m*M* DMOA and 0.1 *M* NaCl at pH 7.15 and 20°C. Separation was investigated at pH values of 7.15, 6.70 and 6 10, at temperatures of 5,

10, 15, 20, 25 and 30°C, with proportions of 0, 0.5, 0 75, 1.0 and 2.0% 2-propanol and with 0, 1, 3, 5 and 10 m*M* DMOA.

Assay II: stereoselective determination of KT, IBU and FENO in human plasma

Analytical procedure. For the determination of the enantiomers in plasma, a 1.00-ml aliquot of KT, or a 0.50-ml aliquot of IBU and FENO, was acidified by adding 0.2 ml of 2 *M* hydrochloric acid, followed by extraction into 6 00 ml of ice-cooled diethyl ether for KT or into 6.00 ml of ice-cooled hexane–diethyl ether (8:2, v/v) for IBU and FENO. After centrifugation (10 min at 1500 *g*), 5.00 ml of the organic layer were removed and evaporated to dryness under a gentle stream of dry nitrogen. Depending on the expected concentrations the residue was redissolved in 0.25 or 0.50 ml of 2-propanol–water (2:8, v/v) and analysed by high-performance liquid chromatography (HPLC).

Standard curves were prepared by injecting plasma extracts spiked with various amounts of the *R*- and *S*-enantiomers of KT and IBU and racemic FENO (enantiomers were not available), simulating the concentrations following oral administration [22–24].

The results of assay I led to a mobile phase composition of 0.5% 2-propanol and 5 m*M* DMOA in 20 m*M* phosphate buffer (pH 6.7) at 15°C for KT, IBU and FENO. The flow-rate was 0.5 ml/min.

Precision of the assay and recovery values. Standard curves with spiked plasma were run five-fold on five consecutive days to determine the day-to-day variability of the method. Recovery values were evaluated by comparing extracted plasma samples with unextracted standard solution in 2-propanol–water (2:8, v/v).

Application. The utility of the method was demonstrated following oral administration of the racemic compounds of KT (100 mg), IBU (600 mg) and FENO (600 mg) to human volunteers. Blood was collected over 10–12 h.

RESULTS

Assay I

The effects of the 2-propanol concentration, pH and temperature on the separation and retention of the racemic compounds of KT, IBU and FENO are summarized in Tables I, II and III. The separation factor could be improved by reducing the concentration of 2-propanol and lowering the temperature, but the retention increased. Table IV illustrates that the higher the content of DMOA the better the enantiomeric resolution. Without DMOA stereoselective separation was either impossible (KT) or unsatisfactory (IBU and FENO). None of the conditions tested was successful in separating the racemic compounds of tiaprofenic acid, indoprofen and carprofen.

Assay II

Fig. 1 shows chromatograms of plasma samples from a volunteer 2 h after oral

TABLE I

EFFECT OF 2-PROPANOL CONCENTRATION ON SEPARATION FACTOR (α), CAPACITY FACTOR (k'_1) AND RESOLUTION FACTOR(R_S)

Mobile phase, 0.02 *M* aqueous phosphate buffer with 5 m*M* DMOA and 0 1 *M* NaCl at pH 7 15 and 20°C.

Concentration of 2-propanol[a] (%)	Ketoprofen			Ibuprofen			Fenoprofen		
	α	k'_1	R_S	α	k'_1	R_S	α	k'_1	R_S
0	1 33	6.72	1.9	1.31	3.96	n.b [b]	1 31	8 79	2.4
0.5	1 30	7.59	1.8	1.29	4.37	n b	1 31	10.31	2.1
0.75	1 23	5 49	1 6	1.23	3 19	n.b	1.23	7.56	1.8
1	1.20	5.09	n.b.	1.23	2.50	n.b.	1.19	7.00	1.7
2	1.13	4.50	n.b.	1.12	2.78	n b	1 13	6 22	n b

[a] No chiral resolution was obtained for KT, IBU and FENO with 5% 2-propanol.
[b] n.b. = no baseline resolution

administration of 100 mg of racemic KT (Fig. 1B), and 4 h after oral administration of 600 mg of racemic IBU (Fig. 1D) and of racemic FENO (Fig. 1E), respectively. Although no FENO enantiomers were available we assume the elution order as given in Fig. 1, because of the stereoselective pharmacokinetic behaviour, particularly the *in vivo* inversion of *R*-FENO to *S*-FENO, as published by Rubin *et al.* [24]. At 4 h after oral administration of racemic FENO we found a higher concentration of peak 6 (Fig. 1E), and 9 h after oral administration only peak 6 was detectable in plasma, so this was therefore assigned to *S*-FENO.

R-IBU could be used as internal standard for KT and FENO; *S*-KT was suitable for IBU. As the use of internal standards gave no better results, quantifi-

TABLE II

EFFECT OF pH ON SEPARATION FACTOR (α), CAPACITY FACTOR (k'_1) AND RESOLUTION FACTOR (R_S)

Mobile phase, 0.5% 2-propanol in 0 02 *M* aqueous phosphate buffer with 5 m*M* DMOA and 0 1 *M* NaCl at 20°C.

pH[a]	Ketoprofen			Ibuprofen			Fenoprofen		
	α	k'_1	R_S	α	k'_1	R_S	α	k'_1	R_S
7 15	1.20	7.59	1.3	1.20	4.37	n b [b]	1.20	12.32	1 6
6 7	1.21	10.36	1 5	1 20	6 20	n b	1.22	14.64	1 7
6.15	1.17	18.40	1 2	1 17	12 03	n b	1.17	26.12	1 6

[a] No chiral resolution was obtained for KT, IBU and FENO with pH values below 6.0
[b] n.b. = no baseline resolution.

TABLE III

EFFECT OF TEMPERATURE ON SEPARATION FACTOR (α), CAPACITY FACTOR (k'_1) AND RESOLUTION FACTOR (R_S)

Mobile phase, 0 5% 2-propanol in 0.02 *M* aqueous phosphate buffer with 5 m*M* DMOA and 0 1 *M* NaCl at pH 7 15.

Temperature (°C)	Ketoprofen			Ibuprofen			Fenoprofen		
	α	k'_1	R_S	α	k'_1	R_S	α	k'_1	R_S
5	1.31	10.88	1 7	1.24	4.51	n b [a]	1.28	15.12	1 6
10	1.29	9.81	1.6	1.23	4 55	n b	1.33	13.32	1 6
15	1.25	8.79	1.5	1.21	4.45	n b	1.27	12 32	1 7
20	1.22	7.97	1 4	1.19	4 53	n.b	1.26	11.07	1.5
25	1.19	7.24	1 3	1 17	4 37	n b	1.22	10 04	1.4
30	1.19	6.65	1.4	1.17	4 30	n b	1 22	9 15	1.5

[a] n.b. = no baseline resolution.

cation was carried out with external standards for the calculation of peak areas. Analytical recovery values and day-to-day variability of the assay are given in Table V. At optimum separation power of the column, depending on its lifetime, 0.1 μg/ml of the enantiomers of KT and IBU in plasma and 0.25 μg/ml of the enantiomers of FENO in plasma could be quantified. Standard curves were linear over the concentration range tested. The correlation coefficients exceeded 0.996. Plasma concentration-time profiles of the *R*- and *S*-enantiomers following oral administration of KT, IBU and FENO are shown in Figs. 2, 3 and 4, respectively.

TABLE IV

EFFECT OF DMOA CONTENT ON SEPARATION FACTOR (α), CAPACITY FACTOR (k'_1) AND RESOLUTION FACTOR (R_S)

Mobile phase, 0.5% 2-propanol in 0.02 *M* aqueous phosphate buffer and 0.1 *M* NaCl at pH 7.15 and 20°C

Concentration of DMOA (m*M*)	Ketoprofen			Ibuprofen			Fenoprofen		
	α	k'_1	R_S	α	k'_1	R_S	α	k'_1	R_S
0	1.00	14.30	n.b.[a]	1.09	7.22	n.b.	1 17	20.29	n b
1	1.12	10.38	n.b.	1.14	5.32	n.b.	1.17	14.59	n b
3	1.15	7.02	n.b	1.15	4.51	n.b.	1 16	10.68	n.b.
5	1.20	11.44	2.0	1.20	5.60	n.b.	1 20	13.61	1 9
10	1.21	13.11	2.3	1.21	7.06	n.b.	1 21	17.43	2 4

[a] n.b. = no baseline resolution

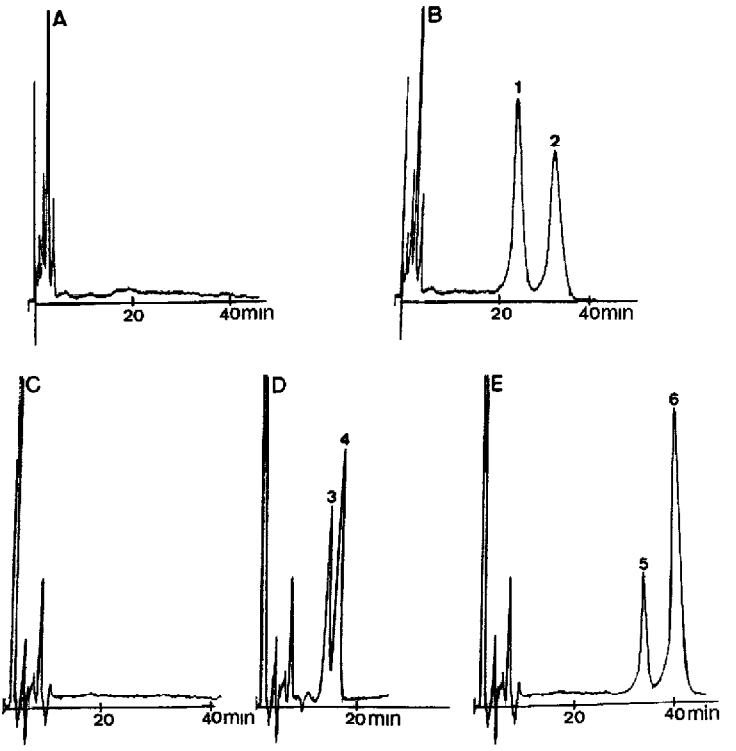

Fig. 1. Chromatograms of blank human plasma (A, detection at 260 nm; C, detection at 220 nm) and of human plasma samples (B, 2 h following oral administration of 100 mg of racemic KT; D, 4 h following oral administration of 600 mg of racemic IBU; E, 4 h following oral administration of 600 mg of racemic FENO) Peaks: 1 = *R*-KT; 2 = *S*-KT, 3 = *R*-IBU; 4 = *S*-IBU, 5 = *R*-FENO; 6 = *S*-FENO No FENO enantiomers were available. The elution order is discussed in the results of assay II.

DISCUSSION

The chiral separation using the EnantioPac column depends to a high degree on chromatographic conditions such as the pH, the ionic strength and content of the organic modifier in the mobile phase, and the temperature. Consequently, the range of optimum conditions to avoid interfering peaks from plasma is rather narrow. The determination of the enantiomers of KT, IBU and FENO in plasma demands a delicate compromise: on the one hand, sufficient enantiomeric resolution and short retention are essential for routine analysis; on the other hand, HPLC conditions have to be chosen to guarantee an acceptable column lifetime. Optimum separation factors for KT, IBU and FENO were achieved with a high DMOA content in the eluent. It is well known, however, that the addition of DMOA to the mobile phase in combination with a high pH damages the column slowly but irreversibly [15]. Consequently, it is obviously necessary to reduce the

TABLE V

RECOVERIES AND DAY-TO-DAY VARIABILITY OF THE ENANTIOMERS OF KT, IBU AND FENO IN PLASMA

Values are mean ± S.D., $n = 5$

Compound	Concentration added (μg/ml)	Concentration found (μg/ml)	Recovery (%)
R-Ketoprofen	0 5	0.51 ± 0.03	103 6 ± 7 9
	1 5	1 56 ± 0.09	98 8 ± 3 9
	2 5	2.46 ± 0 06	105.1 ± 3.0
	5.0	5.01 ± 0.05	101.2 ± 1 9
S-Ketoprofen	0 5	0 53 ± 0.05	108 7 ± 9 2
	1 5	1 56 ± 0.09	98 8 ± 3 9
	2.5	2.46 ± 0.06	105.1 ± 3.0
	5.0	5.01 ± 0 05	101 2 ± 1 9
R-Ibuprofen	0.5	0.51 ± 0.06	102 5 ± 4 7
	2 5	2 53 ± 0 15	96 9 ± 4.2
	5.0	4.93 ± 0.18	97.8 ± 3.8
	10.0	9 94 ± 0 13	100 7 ± 1 3
	20.0	20 14 ± 0 01	100 6 ± 0 6
S-Ibuprofen	0 5	0.52 ± 0 05	106 8 ± 6 7
	2.5	2.38 ± 0 13	98 5 ± 3.2
	5.0	4.89 ± 0.17	101.9 ± 2.8
	10.0	10.04 ± 0 78	93 8 ± 1 3
	20.0	20 08 ± 0 95	103 6 ± 3 8
R-Fenoprofen	0.5	0 52 ± 0 05	104 3 ± 6.9
	2.5	2.45 ± 0.27	93.4 ± 8.0
	5.0	5 16 ± 0 29	98 2 ± 3 9
	10.0	9 81 ± 0 48	99 6 ± 7 8
	25.0	25.06 ± 1 14	99.4 ± 5.4
S-Fenoprofen	0.5	0.54 ± 0.07	108 7 ± 8 9
	2.5	2 45 ± 0 27	93 4 ± 8.0
	5.0	4 76 ± 0 18	98 6 ± 4 7
	10.0	10.15 ± 0 70	101.7 ± 7.7
	25.0	25.21 ± 1 08	99 4 ± 7.7

DMOA content for routine analysis as far as possible Optimum stability of the column was observed using constant chromatographic conditions. As the EnantioPac column is based on immobilized protein it is probable that the separation power may differ from one column to another, requiring minor methodological changes.

Nevertheless, the assay proved to be sufficiently precise and sensitive, as indicated by comparison of the sum of the concentrations of the two enantiomers and the amount of the racemic compound as found by reversed-phase assays (IBU and FENO [25], KT [26]). Consequently, using the described assay it is

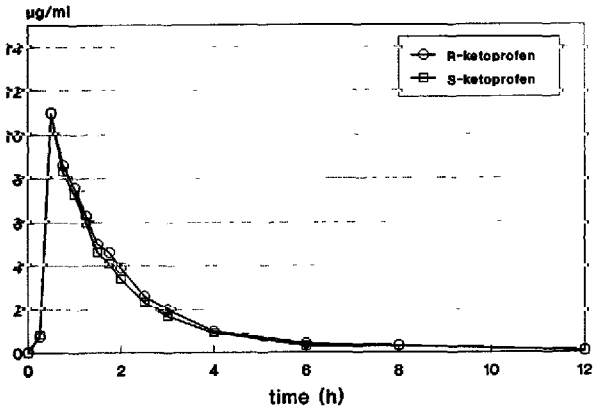

Fig. 2. Plasma concentration–time profiles of *R*-KT and *S*-KT following oral administration of racemic KT (100 mg) to a volunteer The stereoselective pharmacokinetic behaviour of KT is described in detail elsewhere [22,28].

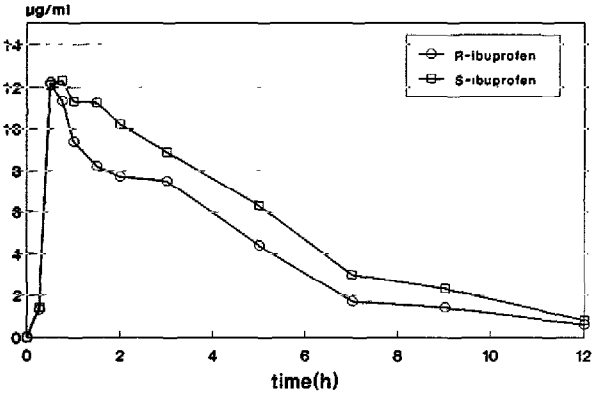

Fig. 3 Plasma concentration–time profiles of *R*-IBU and *S*-IBU following oral administration of racemic IBU (600 mg) to a volunteer. The stereoselective pharmacokinetic behaviour of IBU is described in detail elsewhere [23,27,28].

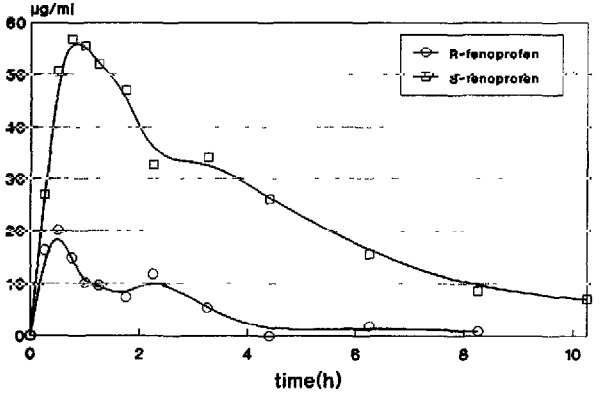

Fig. 4. Plasma concentration–time profiles of *R*-FENO and *S*-FENO following oral administration of racemic FENO (600 mg) to a volunteer The stereoselective pharmacokinetic behaviour of FENO is described in detail elsewhere [24,28].

possible to investigate the pharmacokinetics of KT, IBU and FENO stereoselectively (Figs. 2–4).

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