Journal of Chromatography, 535 (1990) 263–269
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2051

Determination of saterinone enantiomers in plasma samples with an internal standard using a Chiralcel OD column, fractionation and reversed-phase chromatography

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ABSTRACT

A specific and validated high-performance liquid chromatographic method was developed for the determination of the *S*-(−) and *R*-(+) enantiomers of saterinone, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridin-5-yl)phenoxyl]-3-[4-(2-methoxyphenyl)piperazin-1-yl]propan-2-ol, in plasma at the low ng/ml level. The enantiomers of saterinone and an internal standard, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridin-5-yl)phenoxy]-3-[4-(2-ethoxyphenyl)piperazin-1-yl]propan-2-ol, were chromatographed on a chiral Chiralcel OD stationary phase. However, the *S*-(−) enantiomers of saterinone and the internal standard were unresolved, as were the *R*-(+) enantiomers of both substances. Therefore, the two fractions were collected and each was separately resolved on an achiral Polyencap A reversed-phase column and quantified. The detection limit was 0.5 ng/ml of enantiomer, allowing the determination of plasma levels up to 36 h after oral administration of 90, 150 and 180 mg of saterinone to twelve subjects.

INTRODUCTION

Saterinone, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridin-5-yl)phenoxy]-3-[4-(2-methoxyphenyl)piperazin-1-yl]propan-2-ol (A, Fig. 1), is a drug for the treatment of chronic cardiac insufficiency and is administered as the racemate in clinical studies.

To investigate the equivalence of the saterinone enantiomers *in vivo*, a sensitive method is needed to determine plasma levels of the two enantiomers down to the low

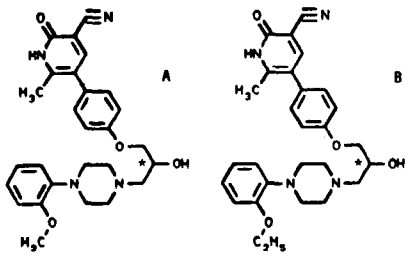

Fig. 1. Structures of (A) saterinone and (B) the internal standard.

0021-9673/90/$03.50

ng/ml level. The determination of racemic saterinone in biological fluids and its direct chromatographic resolution for the determination of the enantiomeric purity of synthesized bulk drugs have been accomplished recently in our laboratory [1,2].

In this paper, a high-performance liquid chromatographic (HPLC) method for the determination of the *S*-(−) (**I**) and *R*-(+) (**II**) enantiomers of saterinone in plasma samples after oral administration of the racemate is described and validated. The pharmacokinetic parameters of the results are presented.

EXPERIMENTAL

Plasma samples

Twelve healthy male subjects, aged 18–40 years, received single oral dose of 90, 150 and 180 mg (3, 5 or 6 tablets, respectively, each containing 30 mg) of saterinone. The medication was administered to the subjects 1 h after breakfast. Plasma samples were collected 0 (prior to administration), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24 and 36 h after administration. All samples were stored in a refrigerator at −85°C until analysed.

Reagents

Perchloric acid (70%), 1 *M* sodium hydroxide solution and methanol of analytical-reagent grade (E. Merck, Darmstadt, F.R.G.) and water purified on a Bion exchanger (Pierce, Rodgau, F.R.G.) were used. *S*-(−)-Saterinone (BDF 9147, CH 1564), *R*-(+)-saterinone (BDF 9144, CH 1516), racemic saterinone (BDF 8634, G 884.2) and the internal standard, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridin-5-yl)phenoxy]-3-[4-(2-methoxyphenyl)piperazin-1-yl]propan-2-ol (BDF 8803 · H_2O · HCl, Roe 956), were synthesized in our Chemical Department.

Equipment

Screw-capped glass vessels (12 ml) with PTFE gaskets from Schott (Mainz, F.R.G.), a vortex Reax-2000 mixer from Heidolph (Kelheim, F.R.G.), a vortex evaporator from Haake–Buchler (Saddle River, NJ, U.S.A.) and an F3 circulation thermostat from Haake Mess-Technik (Karlsruhe, F.R.G.) were used.

Apparatus and HPLC conditions

The HPLC system for the enantiomeric separation consisted of a Model L-6000 pump, a Model F-1000 fluorimeter and a Model D-2500 integrator, all from Merck–Hitachi (E. Merck). The samples were injected with a WISP autosampler (Waters Assoc., Eschborn, F.R.G.) and collected with a Model 202 fraction collector (Abimed, Langenfeld, F.R.G.). A Chiralcel OD (10 μm) column (250 × 4.6 mm I.D.) (Daicel, Tokyo, Japan) with a 0.5-μm eluent filter was used. The column temperature was set to 26 ± 1°C. The eluent was methanol at a flow-rate of 0.5 ml/min. The injection volume was 75 μl.

The fluorimeter was set at an excitation wavelength of 345 nm and an emission wavelength of 435 nm.

Quantification was carried out by using the HPLC system described previously [1]. A Polyencap A (5 μm) column (125 × 4 mm I.D.) (Bischoff Analysentechnik, Leonberg, F.R.G.) with a 20-mm Spherisorb ODS II (5 μm) precolumn was used. The

mobile phase consisted of 1.92 g of sodium 1-pentanesulphonate, 500 ml of water, 300 ml of 0.01 *M* perchloric acid, 300 ml of acetonitrile and 50 ml of tetrahydrofuran. The solution was mixed and filtered through a 0.2-μm PTFE membrane before use. The flow-rate was 0.75 ml/min, the injection volume was 50 μl and the retention times were 3.1 min for **I** and 4.4 min for **II**.

Analytical procedure

To 1 ml of plasma in a screw-capped glass vessel, 50 μl of 1 *M* sodium hydroxide solution were added and extracted with 3 ml of dichloromethane for 15 min. The mixture was centrifuged (2850 *g* for 10 min) and the lower organic phase was transferred to another screw-capped glass vessel and evaporated to dryness. The residue was dissolved in 100 μl of methanol, transferred to an autosampler vial and 75 μl were injected into the HPLC system for fractionation. The two fractions were collected and evaporated to dryness. The residue was dissolved in 75 μl of mobile phase and 50 μl were transferred to an autosampler vial and injected automatically for quantification.

RESULTS AND DISCUSSION

Reproducibility

The day-to-day reproducibility was verified by preparing four calibration graphs. Two analyses were performed for each concentration. No significant difference between the slopes of the four graphs from **I** and **II** was obtained (confidence interval 99% [3]). The results are presented in Table I and show that the method is reproducible.

Linearity

A concentration range of 1.5–50 ng/ml was chosen for the spiked plasma samples, with individual concentrations of 1.5, 2.5, 5, 12.5, 25 and 50 ng/ml each of **I** and **II**. Each analysis was performed four times. The parameters for the resulting

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF SATERINONE ENANTIOMERS IN PLASMA

Each result is the mean of two determinations.

Enantiomer	Date	Slope	Intercept on ordinate	Correlation coefficient
I	08/08/89	19.06	0.40	0.999
	09/08/89	19.16	0.64	0.998
	30/08/89	20.06	0.02	0.998
	31/08/89	19.58	0.40	0.999
II	08/08/89	19.39	0.77	0.999
	09/08/89	20.22	0.36	0.997
	30/08/89	20.05	0.31	0.999
	31/08/89	19.00	0.99	0.998

TABLE II

ACCURACY AND LINEARITY OF THE DETERMINATION OF SATERINONE ENANTIOMERS IN PLASMA

Each result is the mean of four determinations.

Concentration added (ng/ml)	Concentration found (ng/ml)		Deviation from amount added (%)	
	I	II	I	II
1.5	1.70	1.77	+13.3	+18.1
2.5	2.22	2.60	−11.2	+4.0
5.0	4.54	4.21	−9.2	−15.8
12.5	13.18	12.90	+5.4	+3.2
25.0	15.50	26.01	+2.0	+4.0
50.0	49.56	49.36	−0.9	−1.3
		x_{median}	+0.6	+3.6

Parameter	I	II
Intercept on ordinate	0.32	0.48
Slope	19.48	19.71
Correlation coefficient	0.999	0.999

calibration graphs are given in Table II. The calibration graphs were linear for the chosen concentration range.

Accuracy

The data for accuracy were calculated following the requirements given previously [1] and are given in Table II. Four analyses were performed for each concentration. The deviations of the two lowest concentrations were relatively high compared with the others. Minor contaminations in the low concentration range caused higher absolute deviations than those at higher concentrations. However, small matrix effects seemed to be responsible for the higher deviations.

The calculated slopes were 1.003 (**I**) and 1.005 (**II**) and the deviations, x_{median}, were 0.6% (**I**) and 3.6% (**II**). The accuracy of the method was therefore confirmed.

Detection and determination limits

The above limits were calculated as described previously [1]. The detection limit of 0.5 ng/ml and the determination limit of 1.5 ng/ml are identical for both **I** and **II**.

Analytical procedure

An established method for the separation of the enantiomers in bulk drugs [2] proved to be insufficient for the sensitive determination of **I** and **II** in plasma.

To achieve higher sensitivities, the fractions of **I** and **II** were collected and concentrated, after separation on the Chiralcel OD column (Fig. 2a). By using pure methanol, there were no interferences from additives, *e.g.*, buffers or solvents with high boiling points, after gentle evaporation of the eluent. The residues were dissolved and analysed using a second HPLC unit with a reversed-phase column (Fig. 2b), following

the method for the determination of saterinone racemate in biological fluids [1]. The short retention times and sharp peaks obtained resulted in a detection limit down to 0.5 ng/ml of enantiomer in plasma.

To check the work-up procedure, a saterinone analogue with structure and physico-chemical properties both similar to those of saterinone, was chosen as internal standard (B, Fig. 1). The elution profile on the chiral phase was similar to that of saterinone. Both *S*-(−) enantiomers co-eluted and were collected in one fraction, as were the *R*-(+) enantiomers. Quantification was achieved by calculating the ratio of **I** to *S*-(−)-internal standard and of **II** to *R*-(+)-internal standard.

In contrast to preliminary studies, the column temperature was set at 26 ± 1°C in order to obtain shorter retention times with sufficient resolution. The back-pressure of the column increased after analyses of more than 900 samples. Therefore, the column was regenerated by rinsing it in the opposite direction with solvents of different polarity: methanol, methanol–propan-2-ol, propan-2-ol, propan-2-ol–*n*-hexane and *n*-hexane. After this procedure, the back-pressure and the selectivity of the column were the same as those at the beginning of the studies. To check the reproducibility

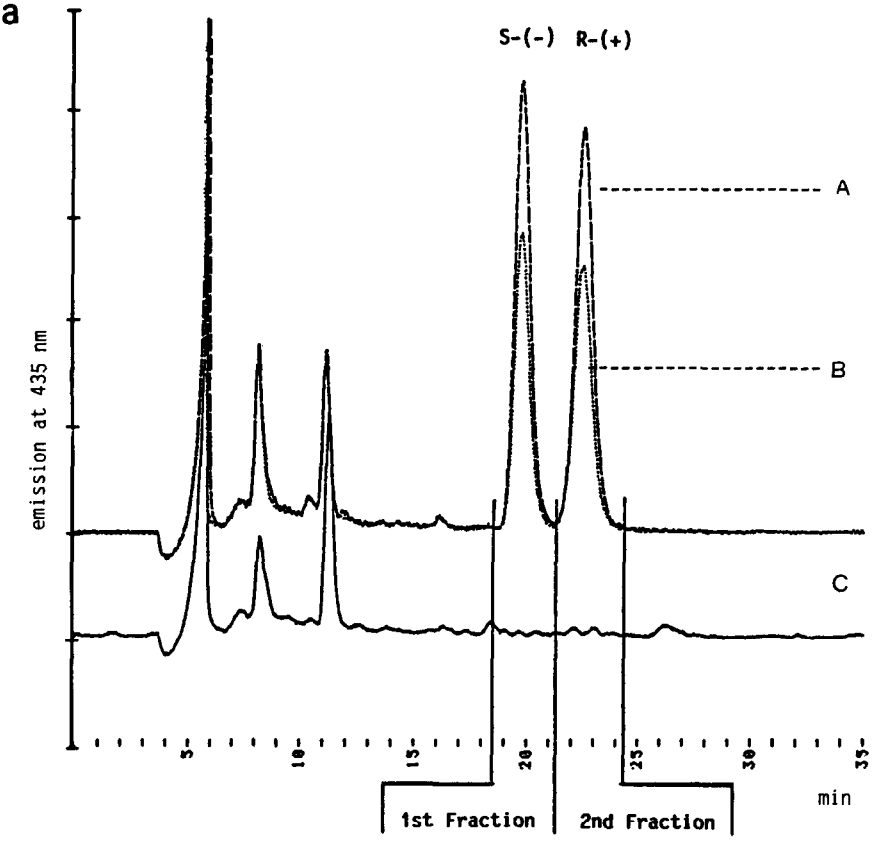

Fig. 2.

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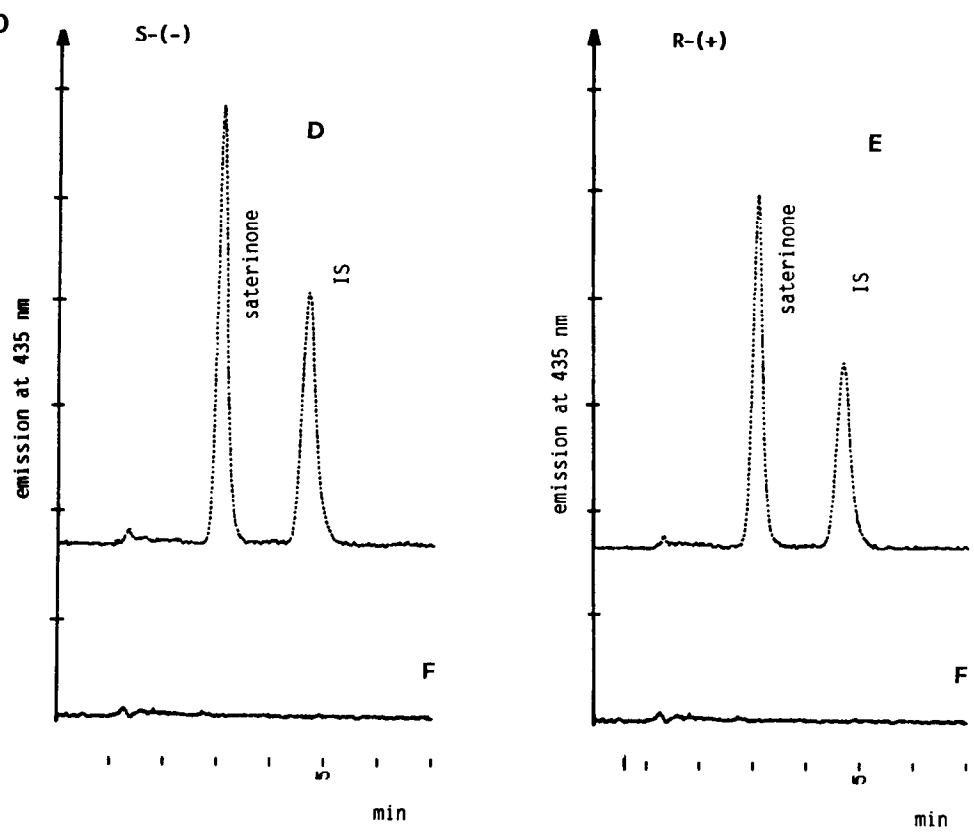

Fig. 2. (a) Chromatograms obtained by injecting 75 μl of (A) plasma sample spiked with 50 ng/ml of saterinone racemate and 50 ng/ml of internal standard, (B) plasma sample spiked with 25 ng/ml of *S*-(−) and 25 ng/ml of *R*-(+) enantiomers of the internal standard and (C) blank. Conditions: 250 mm × 4.6 mm I.D. Chiralcel OD column; eluent, methanol; flow-rate, 0.5 ml/min; temperature, 26°C. (b) Chromatograms obtained by injecting 50 μl of (D) fraction 1, 25 ng/ml of **I** and 25 ng/ml of *S*-(−) enantiomer of the internal standard [see (a)], (E) fraction 2, 25 ng/ml of **II** and 25 ng/ml of *R*-(+) enantiomer of the internal standard [see (a)] and (F) blank. Conditions: 125 mm × 4 mm I.D. reversed-phase Polyencap A column with 20 mm ODS II precolumn; flow-rate, 0.75 ml/min; eluent, 1.92 g of sodium 1-pentanesulphonate salt, 500 ml of water, 300 ml of 0.01 *M* perchloric acid, 300 ml of acetonitrile and 50 ml of tetrahydroforan.

from column to column, a second Chiralcel OD column from a different lot was used. The separation of the enantiomers was well reproduced, showing that the enantiomeric separation was reproducible.

Pharmacokinetic results

The bioequivalence of the enantiomers was studied in twelve volunteers after administration of three different oral doses (90, 150 and 180 mg of saterinone racemate). The elimination half-lives were determined according to the residual technique and the areas under the curves (AUC) were determined by using the trapezoidal rule. The pharmacokinetic results are presented in Table III.

No significant differences for the AUC (0–36 h), AUC (0–∞) and C_{max}

TABLE III

DETERMINATION OF SATERINONE ENANTIOMERS IN PLASMA: MEAN RESULT OF TWELVE DETERMINATIONS

Amount administered (mg)	Parameter	I	II	Ratio (I/II)
90	$t_{1/2}$ (h)	7.5 ± 1.8	9.2 ± 2.5	
	C_{max} (ng/ml)	65.9 ± 24.9	63.6 ± 24.7	1.04 ± 0.05
	AUC (0–t)	208.6 ± 101.8	213.3 ± 93.3	0.98 ± 0.11
	AUC (0–∞)	216.5 ± 106.9	229.8 ± 103.0	0.94 ± 0.10
150	$t_{1/2}$ (h)	7.8 ± 1.6	8.0 ± 1.7	
	C_{max} (ng/ml)	107.2 ± 38.8	106.4 ± 43.3	1.01 ± 0.04
	AUC (0–t)	505.0 ± 178.2	527.8 ± 177.8	0.96 ± 0.08
	AUC (0–∞)	526.5 ± 190.0	552.4 ± 188.6	0.95 ± 0.10
180	$t_{1/2}$ (h)	7.1 ± 1.7	7.9 ± 1.7	
	C_{max} (ng/ml)	115.8 ± 56.0	116.3 ± 54.8	1.00 ± 0.03
	AUC (0–t)	530.5 ± 162.1	573.3 ± 143.0	0.93 ± 0.13
	AUC (0–∞)	545.6 ± 165.4	600.1 ± 159.1	0.91 ± 0.15

parameters between **I** and **II** at the three dose levels were found. These results show that the *S*-(−) and *R*-(+) enantiomers of saterinone are bioequivalent after oral administration of the racemate.

CONCLUSIONS

An HPLC method for the determination of the enantiomeric purity in bulk drugs [2] was improved to determine **I** and **II** in plasma down to the low ng/ml level. The analysis were carried out by using an internal standard with similar structure and chromatographic behaviour for the enantiomeric separation to those of saterinone. The fractionation and quantification were carried out automatically to analyse approximately 40 samples per day.

Because of the low detection limit of 0.5 ng/ml, the plasma samples could be analysed up to 36 h after application. At all three dose levels the pharmacokinetic parameters (AUC 0–∞, AUC 0–t and C_{max}) of **I** and **II** were calculated and tested for their bioequivalence. The statistical evaluation [4] showed no significant differences for the parameters between **I** and **II**.

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