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DIRECT ENANTIOMERIC SEPARATION OF SATERINONE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A new method is described for the direct liquid chromatographic separation and determination of the enantiomers of saterinone, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridinyl-5)-phenoxy]-3-[4-(2-methoxyphenyl)piperazinyl-1]-propan-2-ol, using a Chiralcel OD™ column with methanol as eluent and a temperature set to 10°C. Several lots of synthesized enantiomers were analysed for their enantiomeric purity of more than 99%, because of the excellent resolution ($R_s = 2.2$). Furthermore the enantiomeric ratio was determined in plasma samples after oral and intravenous administration of racemic saterinone.

INTRODUCTION

The racemate of saterinone, 1-[(4-cyano-1,2-dihydro-6-methyl-2-oxopyridinyl-5)-phenoxy]-3-[4-(2-methoxyphenyl)piperazinyl-1]-propan-2-ol (BDF 8634, Fig. 1), a novel drug for the treatment of chronic cardiac insufficiency, is administered in pharmacokinetic studies on laboratory animals and humans [1]. For examination of the pharmacological activity, the *R*-(+) (I) and *S*-(−) (II) enantiomers were synthesized. A sensitive method was employed recently for the determination of saterinone racemate in biological fluids [2].

This paper describes a new method for the direct high-performance liquid chromatographic (HPLC) separation and determination of the enantiomers. Various synthesized lots of I and II exhibited an enantiomeric purity of more than 99%. Human plasma samples were analysed to determine the enantiomeric ratio after intravenous and oral administration of saterinone.

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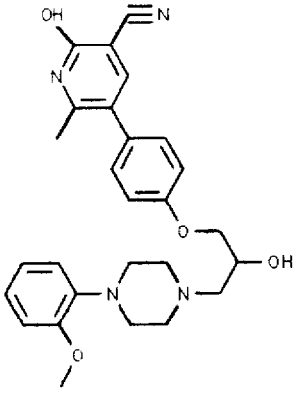

Fig. 1. Structure of saterinone (BDF 8634).

EXPERIMENTAL

Reagents

Methanol LiChrosolv™, 1 *M* sodium hydroxide solution and dichloromethane p.a. were purchased from E. Merck (Darmstadt, F.R.G.). The various saterinone lots of I and II were synthesized in our chemistry department. Drug-free human plasma was obtained from healthy volunteers.

Equipment

Screw-capped glass vials (8 ml) with PTFE gaskets from Schott (Mainz, F.R.G.), a Vortex Reax-2000 mixer from Heidolph (Kelheim, F.R.G.), an Evapotec vortex evaporator and an F3 circulation thermostat both from Haake-Buchler (Saddle River, NJ, U.S.A.) were used. Other instruments were usual laboratory standard.

Apparatus and HPLC conditions

The HPLC system consisted of an L-6000 pump, an F-1000 fluorescence detector and a D-2000 integrator, all from Merck/Hitachi (E. Merck). The fluorimeter was set at an excitation wavelength of 345 nm and an emission wavelength of 435 nm. The samples were injected with a WISP 712 autosampler (Waters, Eschborn, F.R.G.). A Chiralcel OD™ column (250 mm×4.6 mm I.D., 10 μm particle size, Diacel, Tokyo, Japan) with a 0.5-μm eluent filter was used. The column temperature was 10 ± 1 °C. The eluent was methanol with a flow-rate of 0.75 ml/min for plasma and 0.3 ml/min for other samples. The injection volume was 100 μl for plasma and 20 μl for other samples.

Analytical procedure

For the analysis of synthesized lots the substances were dissolved in and diluted with methanol to obtain a concentration of 5 μg/ml and were then analysed directly.

For analysis of plasma samples, 1 ml was pipetted into a screw-capped glass vial and, after addition of 50 μl of 1 *M* sodium hydroxide solution, 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 vial and evaporated to dryness. The residue was dissolved in 100 μl of methanol, transferred to an autosampler vial and injected into the HPLC system.

Calculations

The peak areas of I and II were determined, and the concentrations (ng/ml) of the samples were calculated using a standard curve for I and II.

Recovery

Before each work-up, 1 ml of plasma was spiked with 50 μl of an aqueous solution of different concentrations (see *Linearity*) of each enantiomer. The recovery was determined by comparing the analytical results after work-up with those for the standards without work-up. The mean recovery for I was 66.8% ± 2.9% and for II 65.65 ± 2.8%.

RESULTS AND DISCUSSION

Precision and accuracy

The precision and accuracy are described by the variation of samples spiked with 12.5 ng/ml of both I and II after work-up. The within-assay variability analysed on each of three different days with four samples was 3.2–11.7% for I and 7.5–11.8% for II. The between-assay variability obtained by the mean of the three within-assay calculations was 7.3% for I and 10.1% for II.

Linearity

The concentrations of the spiked plasma were 12.5, 25, 50 and 125 ng/ml for I and II each. Each analysis was carried out four times. The resulting standard curves were linear for the chosen concentration range, which was indicated by the correlation coefficients ($r^2 = 0.9989$ for I and $r^2 = 0.9989$ for II).

Analysis of drug lots

For the separation of the saterinone enantiomers several methods were tested. The derivatization of saterinone with isocyanates, such as 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [3,4] or *S*-(−)-1-phenylethyl isocyanate (PEIC) [5], or with acyl chlorides, such as (+)-1-(9-flu-

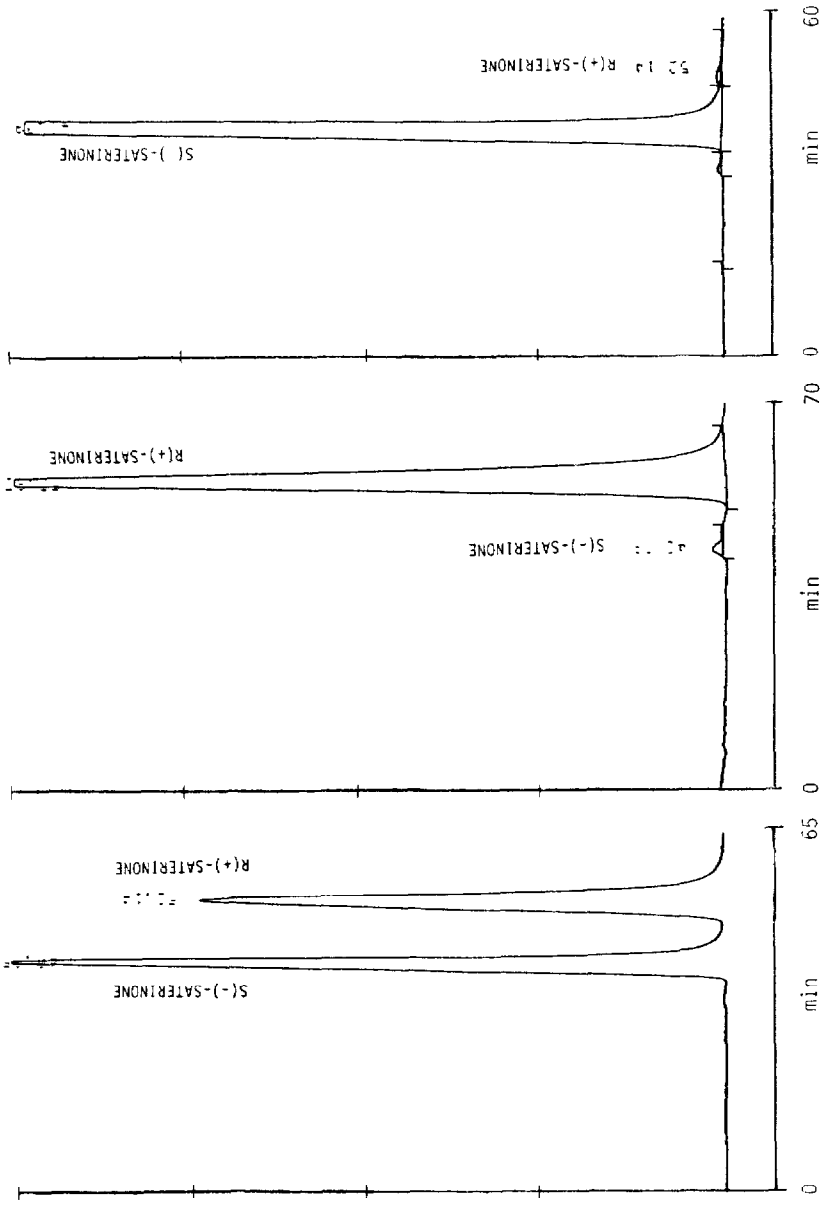

Fig. 2. Chromatograms obtained by injecting 20-μl samples of (A) a standard solution containing 5 μg of racemic saterinone per ml methanol ($\alpha = 1.36$, $R_s = 2.2$), (B) a solution of 5 μg of I (CH 1564) per ml methanol (99.3% enantiomeric purity) and (C) a solution of 5 μg of II (CH 1591) per ml methanol (99.6% enantiomeric purity). Conditions: 250 mm×4.6 mm I.D. Chiralcel OD column; eluent, methanol; flow-rate, 0.3 ml/min; temperature, 10°C.

orenyl)ethyl chloroformate (FLEC) [6], was not successful. In these cases only the side-chain hydroxyl group reacted with the derivatization agents, but not the hydroxyl group of the isopropyl moiety with the optically active carbon atom.

Therefore, direct separation was favoured and investigated. Chiral separations with protein-bonded, optically active polyacrylamide gel or cyclodextrin-bonded phases, described for example by Mehta [7] and Krstulović [8], were not at all successful. However, the use of the tris(3,5-dimethylphenylcarbamate) cellulose column (Chiralcel OD), established by Okamoto and co-workers [9,10], gave excellent results. Krstulović [11] used non-polar eluents to separate the enantiomers of many β-blockers.

In our studies, the separation of saterinone enantiomers with hexane–2-propanol in different proportions was not successful, therefore polar solvents such as ethanol or ethanol–water (Table I) were examined [12]. The two enantiomers were separated with resolution factors from 0.9 (ethanol–water, 2:1; 20°C) to 1.2 (ethanol; 10°C), which was insufficient to determine enantiomeric purities of more than 99%. Even with 2-propanol, long retention times (k' values: S-(−), 6.14; R-(+), 9.86) and insufficient separation were obtained (R_s 1.1). The flow-rate had to be below 0.3 ml/min, because of the instability of the column to pressure (less than 55 bar) and the viscosity of the eluents. When, however, methanol was employed, the resolution was complete. The best results were obtained when the column temperature was 10°C (R_s 2.2) and the flow-rate was 0.3 ml/min (Fig. 2). The low viscosity of methanol resulted in an extended column lifetime owing to the lower back-pressure of 21 bar.

Four lots of the enantiomers I and II were analysed for their enantiomeric

TABLE I

EFFECT OF DIFFERENT POLAR SOLVENTS AND TEMPERATURES ON THE SEPARATION OF I AND II

Flow rate, 0.3 ml/min; injection volume, 50 μl; column, Chiralcel OD 250 mm×4.6 mm I.D.; η = viscosity; k' = capacity factor; α = separation factor; R_s = resolution factor.

Solvent	η (mPa s)	Temperature (°C)	k'_{II}	k'_{I}	α	R_s
Ethanol–water (2:1)	2.34	20	2.71	3.25	1.20	0.9
Ethanol	1.20	30	1.14	1.43	1.25	1.0
		10	2.39	3.18	1.33	1.2
Methanol	0.52	30	0.79	1.11	1.40	1.7
		20	1.35	1.86	1.37	2.0
		10	2.07	2.82	1.36	2.2
2-Propanol	2.27	20	6.14	9.86	1.45	1.1

TABLE II

ENANTIOMERIC PURITY OF I AND II LOTS

Lot	Amount added (%)	Amount analysed (%)	
		II	I
CH 1564	0	0.7	99.3
$S(-)$-Saterinone	1.0	1.5	98.5
	3.0	2.6	97.4
	5.0	3.8	96.2
	10.0	8.5	91.5
$y=a+bx$ ($a=0.54$; $b=0.76$; $r^2=0.994$)			
Extrapolation $y=0$		0.7	99.3
Direct analysis		0.7	99.3
CH 1516	0	99.6	0.4
$R(+)$-Saterinone	1.0	98.9	1.1
	3.0	96.5	3.5
	5.0	94.6	5.4
	10.0	89.9	10.1
$y=a+bx$ ($a=0.33$; $b=0.99$; $r^2=0.999$)			
Extrapolation $y=0$		99.7	0.3
Direct analysis		99.6	0.4
CH 1568	0	0.3	99.7
$R(+)$-Saterinone			
CH 1591	0	99.6	0.4
$S(-)$-Saterinone			

purity (Table II). Quantification of lots CH 1564 and CH 1516 was carried out by applying the standard addition method and by extrapolation to $y=0$. The correlation coefficients of 0.994 and 0.999 indicated that the standard curves were linear. The enantiomeric purities (99.3 and 99.6%) were in good agreement with the values obtained without standard addition (99.3 and 99.7%). Therefore, the other synthesized lots were analysed without standard addition, and comparable purity data were obtained (CH 1568, 99.7%; CH 1591, 99.6%).

Analysis of plasma samples

The results from the determination of the enantiomeric ratio in plasma samples are shown in Table III. The determination limit was 12.5 ng enantiomer per ml plasma, stated after spiking drug-free plasma with saterinone before work-up (Fig. 3). To achieve higher sensitivity, the eluent flow-rate was increased to 0.75 ml/min despite the lower resolution (R_s 1.6) that this caused. Only a few samples with high saterinone content have been analysed so far, because the determination of the enantiomers was not as sensitive as the determination of the racemate (determination limit 0.75 ng/ml). Saterinone racemate was administered to four volunteers. The dosage scheme was 2 μg/

TABLE III

ENANTIOMERIC RATIO OF I AND II IN PLASMA SAMPLES AFTER ADMINISTRATION OF SATERINONE RACEMATE

Volunteer	Time point (min)	Racemate concentration (ng/ml)	Amount analysed (%)	
			I	II
Intravenous administration of 2 and 4 μg/kg body weight per min				
A	21	198.2	45.3	54.7
	25	215.0	48.8	51.2
B	21	183.5	48.7	51.3
	25	187.0	48.5	51.5
Oral administration of 60 mg and additional 30 mg after 2 h				
C	180	90.9	53.1	46.9
	240	65.7	48.3	51.7
D	180	125.5	48.3	51.7
	240	109.5	50.2	49.8

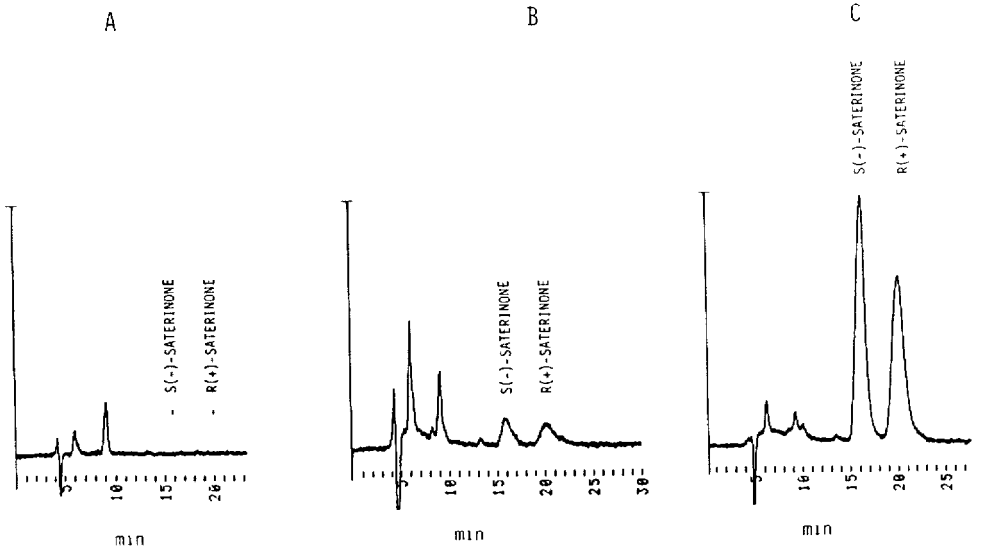

Fig. 3. Chromatograms of (A) a blank after work-up of 1 ml of drug-free plasma, (B) a plasma sample spiked with 25 ng of racemic saterinone per ml and (C) a plasma sample from a volunteer. The concentration is 187.0 ng of racemic saterinone per ml (48.5% I and 51.5% II). For dosage details see text.

kg body weight per minute from the start of infusion to the tenth minute, and another 4 μg/kg body weight per minute from the fifteenth to the twenty-fifth minute ($n=2$). The oral dose was 60 mg at the start and another 30 mg after

2 h ($n=2$). Plasma was collected 21 and 25 min after intravenous and 180 and 240 min after oral administration. The concentrations of racemic saterinone were determined by the method described in the literature [2]. Nevertheless, no significant differences between the plasma levels of the two enantiomers were found, indicating comparable metabolism of saterinone enantiomers. In all cases the enantiomeric ratio was close to 1, after both oral and intravenous administration. To verify the preliminary results further investigations with samples from present studies will be carried out.

CONCLUSIONS

A method for the direct chromatographic separation of saterinone enantiomers was developed. In contrast to other applications, several polar solvents were proved to be useful as mobile phases with a Chiralcel OD column. Owing to the low viscosity of methanol at 10 °C and good solubility of most drugs it is an excellent eluent. The column lifetime was tested for ca. ten months, without any deterioration of resolution. Purities of more than 99% could be checked for both enantiomers. Further investigations will be carried out to optimize the determination method of the enantiomers in plasma, to achieve higher sensitivity and to obtain more information about pharmacokinetic parameters.

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