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Short Communication

Direct separation of the enantiomers of propafenone, diprafenone and their major metabolites by high-performance liquid chromatography on modified cellulose and amylose chiral stationary phases☆

Th. Hollenhorst and G. Blaschke*

Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstrasse 58–62, 4400 Münster (Germany)

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ABSTRACT

The enantiomers of propafenone, diprafenone and their major metabolites, N-desalkylpropafenone, 5-hydroxypropafenone and 5-hydroxydiprafenone, were separated on a cellulose tris-3,5-dimethylphenylcarbamate column (Chiralcel OD) and an amylose tris-3,5-dimethylphenylcarbamate column (Chiralpak AD).

INTRODUCTION

The chiral antiarrhythmic drug propafenone (Fig. 1) is used in therapy as the racemate. The sodium antagonistic acitivity of the enantiomers of propafenone is about equal [1]. However, owing to the partial structure of a β-blocking agent, the enantiomers differ in their affinity for the β-adrenergic receptor. Thus, *S*-(+)-propafenone is a hundred times more potent at the β-receptor than *R*-(−)-propafenone [1]. The metabolites N-desalkylpropafenone and 5-hydroxypropafenone (Fig. 1) have similar electrophysiological effects as the parent drug but their β-adrenergic activity is negligible [2]. The increase in the plasma levels of propafenone may cause serious β-adrenergic side-effects as a result of the *S*-(+)-enantiomer. Poor metabolizers lacking the cytochrome P-450 isoenzyme which catalyzes the metabolic conversion of the drug are particularly susceptible to these adverse reactions. Side-effects may also occur in extensive metabolizers when high initial doses are given because the hydroxylating P-450 enzyme system is saturable. Thus, a simple assay for the monitoring of the enantiomeric ratio of propafenone in body fluids is required.

The enantiomers of propafenone have been prepared [3,4]. Circular dichroism (CD) measurements assigned the *R*-configuration to the levorotatory base and the dextrorotatory hydrochloride salt [3,4]. The configuration has recently been confirmed by X-ray analysis [5]. Following derivatization with optically pure isocyanates the resulting diastereomers can be separated by high-performance liquid chromatography (HPLC) [3,4].

☆ Dedicated to Professor Dr. Dr. E. Mutschler on the occasion of his 60th birthday.

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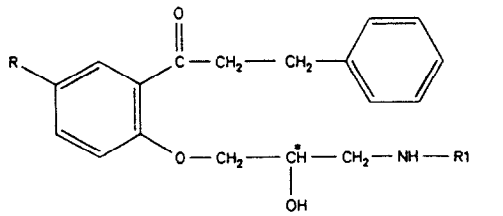
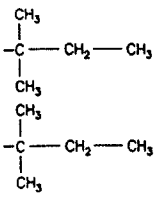


Propafenone R=H ; R1 = $-CH_2-CH_2-CH_3$
N-desalkylpropafenone R=H ; R1 = H
5-hydroxypropafenone R=OH ; R1 = $-CH_2-CH_2-CH_3$



Diprafenone R=H ; R1 = $-C(CH_3)_2-CH_2-CH_3$

5-hydroxydiprafenone R=OH ; R1 = $-C(CH_3)_2-CH_2-CH_3$

Fig. 1. Structures of compounds.

In this communication we would like to describe the first direct resolution of the enantiomers of propafenone, diprafenone and their major metabolites (Fig. 1) on a cellulose tris-3,5-dimethyl–phenylcarbamate column (Chiralcel OD) and an amylose tris-3,5-dimethylphenylcarbamate column.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Beckman 110B solvent-delivery module (Beckmann, Fullerton, CA, USA), a Lambda 1000 detector (Bischoff, Munich, Germany) operated at 254 nm if not stated otherwise and a CR3-A Chromatopac integrator (Shimadzu, Columbia, MD, USA). A Chiralcel OD column and a Chiralpak AD column containing cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel and amylose tris-3,5-dimethylphenylcarbamate coated on silica gel (each 25 cm × 0.46 cm I.D. particle size 10 μm, both purchased from J. T. Baker, Gross-Gerau, Germany) were used.

Chemicals

Propafenone and its metabolites were obtained from Knoll (Ludwigshafen, Germany). The enantiomers of propafenone were prepared as previously published [3,4]. Diprafenone were purchased from Helopharm (Berlin, Germany). *n*-Hexane, 2-propanol, 2-butanol and diethylamine were purchased from Merck (Darmstadt, Germany) in the highest purity available.

Chromatographic conditions

The concentrations of the compounds were 20 μg/ml in *n*-hexane–2-propanol (90:10) (propafenone and diprafenone) or 2-propanol (hydrophilic metabolites). A 100-μl aliquot was injected into the HPLC system. For mobile phase compositions see Table I.

Preparation of blood samples

To 1.0 ml of plasma were added 200 μl of a *M* sodium hydroxide solution to give pH $\geqslant$ 12. Following addition of 5.0 ml of dichloromethane, the samples were mixed for 10 min and centrifuged for 10 min at 2500 *g*. The organic layer was transferred into a clean glass tube and evaporated under a stream of nitrogen. The residue was dissolved in 100 μl of hexane–2-propanol (90:10) and injected into the chromatographic system B (see Table I).

RESULTS AND DISCUSSION

The direct separation of the enantiomers of β-blocking drugs has been successfully accomplished on a Chiralcel OD column [6]. Thus, this chiral stationary phase was also applied for the resolution of propafenone and its metabolites. The results are summarized in Table II. The use of the standard mobile phase of the Chiralcel OD column, *n*-hexane–2-propanol (90:10), resulted in only an incomplete separation of the enantiomers which was unsuitable for quantitative analysis. Complete resolution was achieved by substituting 2-butanol for 2-propanol. Moreover, the concentration of the modifier was reduced to 7%. The addition of diethylamine suppressed the tailing of the peaks.

The best results for the metabolite N-desalkylpropafenone were obtained by increasing the 2-butanol content of the eluent to 15%. Additionally, the diethylamine concentration was reduced (Fig. 2b). No separation of the enantiomers of 5-hydroxypropafenone was achieved using an alkaline eluent.This can be explained by the fact that the phenolate anion which is formed in alkaline media inhibits sufficient interaction of the enantiomers

TABLE I

MOBILE PHASES

System	*n*-Hexane	2-Butanol	2-Propanol	Diethylamine	Acetic acid	Flow-rate (ml/min)
A	93	7	0	0.2	0	1.0
B	75	0	25	0.2	0	1.0
C	75	0	25	0.2	0	1.5
D	85	15	0	0.1	0	2.0
E	80	20	0	0	0.85	1.0
F	90	0	10	0	0	1.0
G	84	0	16	0	0	1.0

with the chiral stationary phase. In contrast, good resolution resulted under acidic conditions, with the addition of 0.85% acetic acid instead of diethylamine. The concentration of the acid has to be kept within tight limits because small changes cause a significant loss of the resolution of the enantiomers. Trichloroacetic acid was not superior to acetic acid.

The analogue diprafenone and its major metabolite 5-hydroxydiprafenone were resolved under similar conditions (Fig. 2c and Tables I and II). In comparison with the Chiralcel OD column the use of a Chiralpak AD column resulted in an improvement of the resolution of propafenone and a simultaneous reduction in the retention times (Fig. 2a) and Tables I and II). With the exception of diprafenone the other compounds could not be separated on this stationary phase. The separation of the enantiomers of propafenone and N-desalkylpropafenone and unresolved 5-hydroxypropafenone in a single run was achieved by directly connecting the Chiralcel OD and the Chiralpak AD columns (Fig. 2d).

Both chiral stationary phases have been used on a daily basis for more than one year under the conditions described without any loss of performance.

The assay described is suitable for the analysis of the enantiomeric ratio of propafenone in body fluids. Fig. 3 shows representative chromatograms of plasma samples. The stereoselective metabolism of propafenone has been demonstrated using an indirect method after derivatization with isothiocyanates [1]. In accordance with this report the stereoselective metabolism of propafenone can also be shown with the direct method of the separation of the enantiomers of propafenone (Fig. 3c).

TABLE II

SEPARATION OF RACEMATES

Racemate	Column	System	Capacity factors		Selectivity (α)	Resolution factor (R)
			k'_1	k'_2		
Propafenone	OD	A	6.45	8.05	1.24	2.16
Propafenone	AD	A	5.78	7.9	1.36	3.59
Propafenone	AD	B	1.57	2.81	1.79	5.56 (Fig. 2a)
Propafenone	AD	C	1.45	2.54	1.75	4.35
N-Desalkylpropafenone	OD	D	1.0	5.4	5.4	10.13 (Fig. 2b)
5-Hydroxypropafenone	OD	E	5.75	8.25	1.43	1.0
Diprafenone	OD	F	2.12	3.75	1.77	4.72 (Fig. 2c)
5-Hydroxydiprafenone	OD	E	4.3	5.8	1.35	1.28

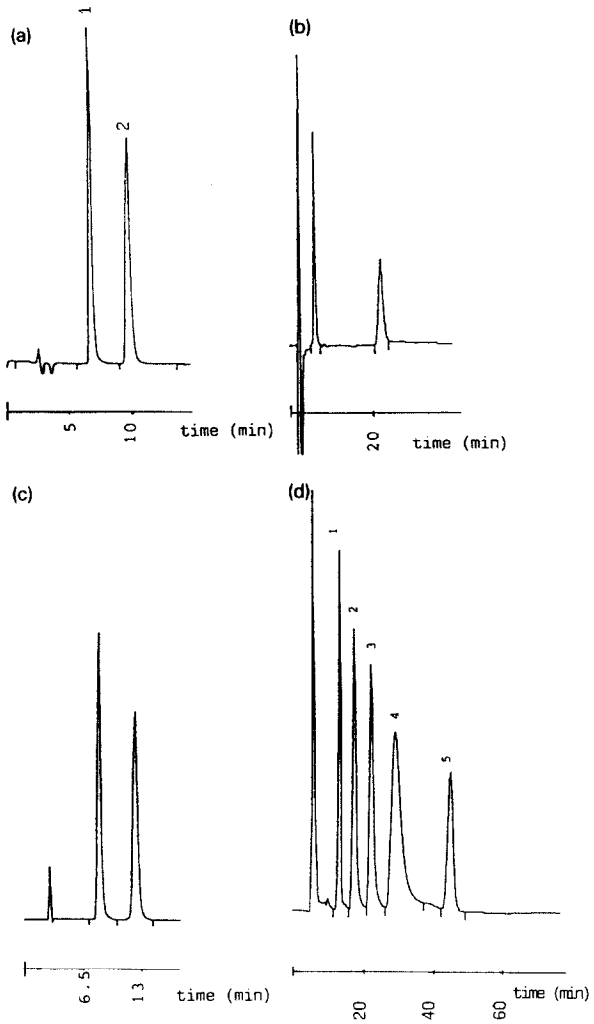


Fig. 2. Chromatograms of separations of the racemates. (a) Separation of propafenone on Chiralpak AD, mobile phase system B: peak 1 = *R*-(−)-propafenone; peak 2 = *S*-(+)-propafenone. (b) Separation of N-desalkylpropafenone on chiralcel OD, mobile phase system D. (c) Separation of diprafenone on Chiralcel OD, mobile phase system F. (d) Mixture of racemic propafenone, N-desalkylpropafenone and 5-hydroxypropafenone, mobile phase system G; column, Chiralcel OD and Chiralpak AD directly connected; wavelength of detection, 222 nm; peaks 1 and 5 = N-desalkylpropafenone; peak 2 = -*R*-(−)-propafenone; peak 3 = *S*-(+)-propafenone; peak 4 = unresolved 5-hydroxypropafenone. For all chromatograms 2 μg of the compounds were injected. For the compositions of the mobile phases see Table I.

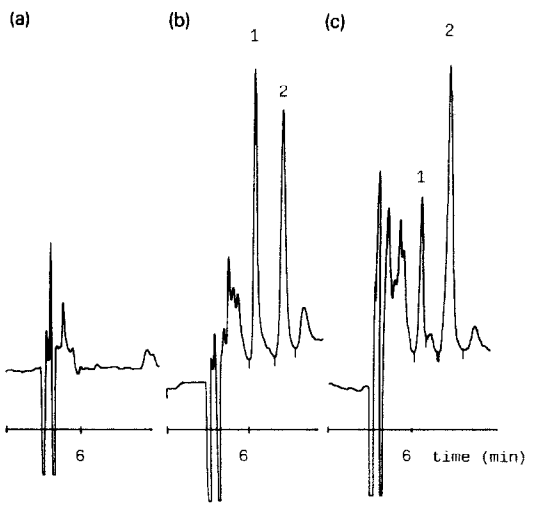


Fig. 3. Chromatograms of plasma samples. (a) Blank plasma; (b) blank plasma spiked with 200 ng/ml of each enantiomer of propafenone; (c) sample 2 h after oral administration of 300 mg of racemic propafenone to humans. Peaks: 1 = *R*-(−)-propafenone; 2 = *S*-(+)-propafenone. Chromatographic conditions: mobile phase, *n*-hexane–2-propanol–diethylamine (75:25:0.2); flow-rate, 1.0 ml/min; column, Chiralpak AD.

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