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Enantioselective analysis of propafenone in plasma using a polysaccharide-based chiral stationary phase under reversed-phase conditions

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Abstract

We present a method for the enantioselective analysis of propafenone in human plasma for application in clinical pharmacokinetic studies. Propafenone enantiomers were resolved on a 10-μm Chiralcel OD-R column (250×4.6 mm I.D.) after solid-phase extraction using disposable solid-phase extraction tubes (RP-18). The mobile phase used for the resolution of propafenone enantiomers and the internal standard propranolol was 0.25 *M* sodium perchlorate (pH 4.0)–acetonitrile (60:40, v/v), at a flow-rate of 0.7 ml/min. The method showed a mean recovery of 99.9% for (*S*)-propafenone and 100.5% for (*R*)-propafenone, and the coefficients of variation obtained in the precision and accuracy study were below 10%. The proposed method presented quantitation limits of 25 ng/ml and was linear up to a concentration of 5000 ng/ml of each enantiomer. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Propafenone

1. Introduction

Propafenone (2′-[2-hydroxy-3-(propylamino)-propoxy]-3-phenylpropiophenone, Fig. 1) is a potent type Ic antiarrhythmic agent that is effective against supraventricular and ventricular arrhythmias [1–4]. Propafenone is administered as a racemic mixture of (*S*)- and (*R*)-enantiomers. Although both enantiomers are equally potent in their activity as sodium channel blockers, the (*S*)-enantiomer exhibits β-blocking activity aproximately 100 times higher [5,6].

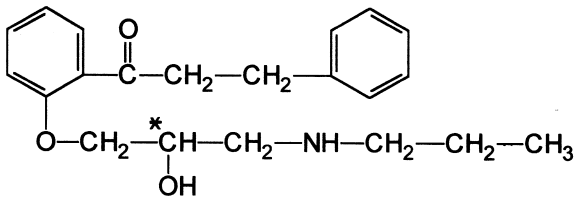

Fig. 1. Structure of propafenone; * denotes the chiral center.

Both enantiomers are extensively metabolized to 5-hydroxypropafenone, which also exhibits antiarrhythmic properties in vivo [7]. This metabolism is polymorphic and correlates with the functional presence or absence of the hepatic cytochrome P-450 IID6. This polymorphic metabolism is of clinical importance because the higher plasma propafenone concentration seen in poor metabolizers may lead to an increased risk of side effects on the central

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nervous system as well as clinically evident β-blockade [8]. The enantiomers of propafenone interact with respect to P450 IID6-mediated 5-hydroxylation with (*R*)-propafenone being a more potent inhibitor than (*S*)-propafenone. These findings suggest that the administration of (*R*)-propafenone rather than racemic propafenone may be of advantage in β-blockade-intolerant patients [9–11].

The stereoselective differences in the pharmacodynamic and pharmacokinetic properties of propafenone justify the development of methods for the separation and determination of the enantiomers in plasma samples. The resolution of propafenone enantiomers by HPLC has been performed by indirect methods involving derivatization with chiral reagents [5,12] or by direct methods which employ chiral mobile phases [13,14] or chiral stationary phases [15–17].

Chiral separation of propafenone has been performed successfully under normal-phase condition, using chiral stationary phases based on polysaccharide derivatives. Hollenhorst and Blaschke [15] separated the enantiomers of propafenone and *N*-desalkylpropafenone and (*R,S*)-5-hydroxypropafenone using the Chiralcel OD and Chiralpak AD columns connected in series. In 1993, Aboul-Enein and Bakr [16] reported a method for the separation of propafenone enantiomers and (*R,S*)-5-hydroxypropafenone using a Chiralcel OD column. The Tris–3,5-dimethylphenyl carbamate derivative of amylose (Chiralpak AD column) was used by Bohm et al. [17] for the determination of (*R*)- and (*S*)-propafenone as well (*R,S*)-5-hydroxypropafenone in plasma samples after liquid–liquid extraction of the drugs with dichloromethane. The method described showed limits of quantitation of 10 ng/ml for both enantiomers of propafenone and of 20 ng/ml for (*R,S*)-5-hydroxypropafenone.

The Tris–3,5-dimethylphenyl carbamate derivative of cellulose can be used also under reversed-phase conditions. This column has not been used for the resolution of propafenone enantiomers, in spite of the advantage of being used with an aqueous–organic mobile phase. Therefore, the commercial column Chiralcel OD-R was used in this study for the development of a method for the determination of propafenone enantiomers in plasma samples.

2. Experimental

2.1. Standard solutions and chemicals

A stock solution of (*R,S*)-propafenone·HCl, kindly supplied by Knoll S.A., Brazil, was prepared at a concentration of 1.0 mg free base/ml, in methanol acidified with 0.01 *M* hydrochloride acid. Working solutions at concentrations of 2.0, 4.0, 8.0, 20.0, 40.0, 200.0 and 400.0 μg free base/ml were prepared by appropriate dilution. The solutions were stored at 4°C and were stable for at least 2 months. A (*R,S*)-propranolol·HCl solution used as the internal standard was prepared in methanol at a concentration of 100.0 μg/ml.

The solvents used as mobile phase or in the extraction procedure were chromatography grade (Merck, Darmstadt, Germany). Other reagents were of analytical grade (Merck, Carlo Erba, Milan, Italy)

2.2. Apparatus and chromatographic conditions

The equipment used was a Shimadzu (Kyoto, Japan) liquid chromatography apparatus consisting of a Model LC-10AS solvent pump, a Model 7125 Rheodyne injector with a 20-μl loop, a Model SPD-10 A variable-wavelength UV detector operating at 300 nm and a Model CR6-A integrator.

Propafenone enantiomers were resolved using a 10-μm Chiralcel OD-R column (250×4.6 mm I.D., Chiral Technologies, Exton, USA) protected with a 4×4 mm RP-8 endcapped precolumn (Merck, Darmstadt, Germany) and a mobile phase consisting of 60% 0.25 *M* sodium perchlorate added with perchloric acid to pH 4.0 and 40% acetonitrile at a flow-rate of 0.7 ml/min. The separations were carried out in a thermostated room with temperature set at 25°C.

2.3. Extraction procedure

Aliquots of 1 ml of plasma samples pooled from healthy volunteers were spiked with 25 μl of internal standard solution ((*R,S*)-propranolol, 100 μg/ml) and were applied to disposable solid-phase extraction tubes (ENVI-18, 500 mg, 6 ml, Supelco, Bellefonte, USA), previously conditioned by the elution of 2 ml

methanol and 2 ml water. The samples were passed slowly through the extraction tube under atmospheric pressure and the columns were then washed with 5 ml water. The residual water was eliminated by eluting 1 ml methanol and the columns were dried under vacuum for 10 min. Propafenone and internal standard were eluted using 2 ml of a methanol solution of ammonium hydroxide (0.7 *M*) into conical polypropylene tubes. The solvent was evaporated dry under an air flow at room temperature. The residue thus obtained was dissolved in 100 μl of the mobile phase and 100 μl of hexane. After vortex-mixing for 1 min and centrifugation for 5 min (1800 *g*), 20 μl of the lower phase were submitted to chromatographic analysis.

For the construction of the calibration curves, 25 μl of the propafenone solution at concentrations of 8.0–200.0 μg/ml and 25 μl of the propranolol solution (internal standard) at a concentration of 100.0 μg/ml were added to test tubes, the solvent was evaporated under an air flow at room temperature, and the residues were supplemented with 1 ml blank plasma and extracted as described earlier.

2.4. Assay specification

To determine absolute recovery, plasma samples containing propafenone in the concentration range of 100–2500 ng/ml of each enantiomer (100, 250, 500 and 2500 ng/ml) were extracted in triplicate by the procedure proposed. The eluate obtained was supplemented with 25 μl of propranolol solution and, after evaporation of the solvent, the residues were submitted to partition wih 100 μl of the mobile phase and 100 μl of hexane and chromatographed. The concentrations of these samples were determined on the basis of the calibration curve obtained with the data for the drug not submitted to extraction. Linearity was assessed in the 100–5000 ng/ml range for each enantiomer.

The precision and accuracy of the method were tested in within-day and between-day studies. For the determination of within-day precision and accuracy, aliquots ($n=10$) of plasma enriched with propafenone at concentrations of 200 and 2000 ng/ml of each enantiomer were analyzed according to the procedure described earlier, and the results obtained were expressed as relative standard deviation (coefficient of variation, C.V.). The between-day precision and accuracy were calculated by analyzing aliquots of the same samples on five consecutive days. In plasma samples obtained after oral administration of propafenone to EM metabolizers the concentration of the (*S*)-enantiomer is higher than that of the (*R*)-enantiomer [5,10], and to evaluate the precision and accuracy of the method under such conditions the within-day precision and accuracy were determined in a sample spiked with approximately 100 ng/ml of the (*S*)-enantiomer and 50 ng/ml of the (*R*)-enantiomer ($n=5$). In this experiment, a standard solution of *rac*-propafenone was injected into a Chiralpak AD column, according to the method described by Bohm et al. [17]. The individual enantiomers were collected after eluting from the column, and the mobile phase was evaporated dry under an air flow. The residues were dissolved in methanol and these solutions were used to spike plasma samples with the individual enantiomers or with both enantiomers, with the concentration of (*S*)-propafenone being twice that of the (*R*)-enantiomer in the latter case.

The quantitation limit was assessed by analyzing aliquots ($n=4$) of plasma spiked with propafenone at a concentration of 25 ng/ml of each enantiomer. To assess the selectivity of the method, several drugs that can be administered in combination with propafenone were submitted to chromatographic analysis at concentrations corresponding to the maximum value of the therapeutic range. The drugs whose retention times were similar to those of the propafenone enantiomers or of the internal standard were added to 1-ml aliquots of the blank plasma and submitted to the extraction procedure before chromatographic analysis.

3. Results and discussion

The resolution of propafenone enantiomers on a Chiralcel OD-R column has not been described until now, although this column has the advantage of being used with aqueous–organic mobile phases [18]. Using a perchlorate–acetonitrile mixture it was possible to obtain a separation factor of 1.07 and elution times shorter than 20 min. The resolution

could not be improved by changing pH or concentration of perchlorate solution; the amount of acetonitrile was established as 40% in order to reduce the analysis time. The elution order was determined by the analysis of individual enantiomers, previously separated and collected from the Chiralpak AD column, using the mobile phase hexane–isopropanol (83.4:16.6, v/v) supplemented with 0.25% diethylamine, according to the method described by Bohm et al. [17]. The elution order obtained under reversed-phase conditions was inverse in relation to normal-phase conditions [15,17]. Under the conditions employed, the metabolites of propafenone elute with a retention time of 8.6 min for 5-hydroxypropafenone and 8.3 for nor-propafenone, without interfering with the analysis (Fig. 2).

Fig. 3 shows typical chromatograms obtained after solid-phase extraction of blank plasma and blank plasma to which the internal standard propranolol and propafenone had been added. The extraction procedure involved the use of solid-phase cartridges

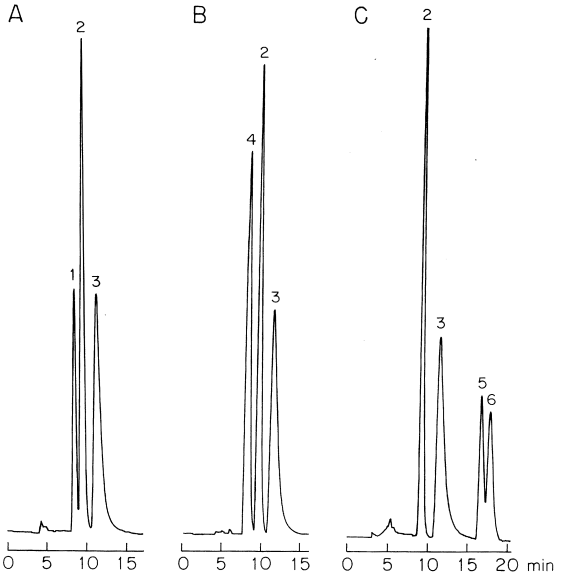

Fig. 2. Chromatograms showing the resolution of propafenome, propranolol and propafenone metabolites on the Chiralcel OD-R column. (*R*,*S*)-5-Hydroxypropafenone (1), propranolol enantiomers (2,3), (*R*,*S*)-nor-propafenone (4), (*S*)-propafenone (5) and (*R*)-propafenone (6). Chromatographic conditions: Chiralcel OD-R column, 10-μm particles (4.6×250 mm I.D.); sodium perchlorate 0.25 *M* (pH 4.0)–acetonitrile (60:40, v/v) as mobile phase at a flow-rate of 0.7 ml/min; detection at 300 nm.

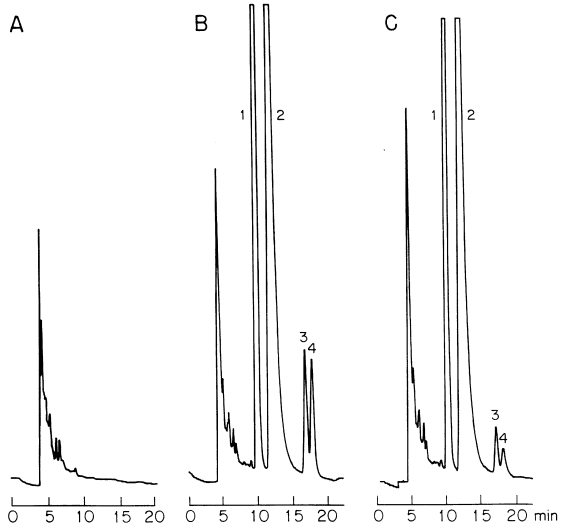

Fig. 3. Chromatograms referring to the analysis of propafenone enantiomers in plasma. (A) Blank plasma; (B) plasma spiked with 250 ng/ml of the enantiomers (*S*)-propafenone (3) and (*R*)-propafenone (4) and propranonol enantiomers (1,2); (C) plasma spiked with 100 ng/ml of the enantiomers (*S*)-propafenone (3) and 50 ng/ml of (*R*)-propafenone (4) and propranolol enantiomers (1,2). Chromatographic conditions were the same as in Fig. 2.

packed with 500 mg of C_{18} bonded phase. After the washing procedure with 5 ml water and 1 ml methanol, the drugs were eluted using a methanol–ammonium hydroxide solution. It was necessary to add ammonium hydroxide to the elution solvent in order to avoid the irreversible retention of propafenone in the residual silanol groups. Some low-polarity endogenous substances extracted during solid-phase extraction could not be dissolved by the mobile phase. To prevent this particulated material from entering the column, a further partition step with hexane was introduced before chromatographic analysis [19]. This extraction procedure and the selection of 300 nm as the wavelength for detection were highly efficient in removing endogenous interferents. Recoveries of 99.9% for (*S*)-propafenone and of 100.5% for (*R*)-propafenone were obtained, regardless of concentration within the range studied.

To select a suitable internal standard, several drugs were analyzed under the established conditions. Propranolol was selected because it is a structural analog of propafenone and could be extracted with reproducibility under the conditions

employed. Propranolol enantiomers could be resolved under the conditions employed and therefore the first peak was used in the quantitation because it was more symmetrical than the second one. Fig. 3 shows also the chromatogram of a plasma sample spiked with different amounts of propafenone enantiomers. Different amounts of propafenone enantiomers could also be observed in the chromatogram (Fig. 4) of a sample collected from a healthy volunteer after the administration of a single dose of (*R*,*S*)-propafenone (Ritmonor, 150 mg).

Peak heights of (*S*)-propafenone and (*R*)-propafenone correlated linearly with concentration of both enantiomers in the range of 100–5000 ng/ml (correlation coefficients of 0.9937 and 0.9997, respectively). Typical calibration curves obtained by least-squares linear regression were $19.236x+0.894$ and $17.179x+0.564$ for the (*S*)- and (*R*)-enantiomers, respectively.

The within-day precision and accuracy (from nominal values) of the assay (Table 1) were determined by subsequential analysis of multiple spiked sample of *rac*-propafenone in human plasma ($n=10$). Aliquots of the same samples were analysed on five consective days for the evaluation of between-day precision and accuracy. At both concentration levels tested the coefficients of variation for precision and accuracy were below 10%.

Similar results for within-day precision and accuracy were observed by the analysis of the sample spiked with different amounts of the individual enantiomers obtained after separation on the Chiralpak AD column (Table 2). The real concentration of these samples was considered to be the concentration obtained by the analysis of the samples spiked with the individual enantiomers. In this study it was also possible to observe no racemization of propafenone during sample workup.

The quantitation limit is a parameter used for the measurement of the sensitivity of the method and is defined as the concentration whose accuracy and precision are less than 10% [20]. In the present study, the quantification limit determined from extracted spiked plasma ($n=4$) was 25 ng/ml for both propafenone enantiomers (Table 1). Although this quantitation limit is higher than the limit obtained by Bohm et al. [17], it is enough for application of the method to clinical pharmacokinetic studies.

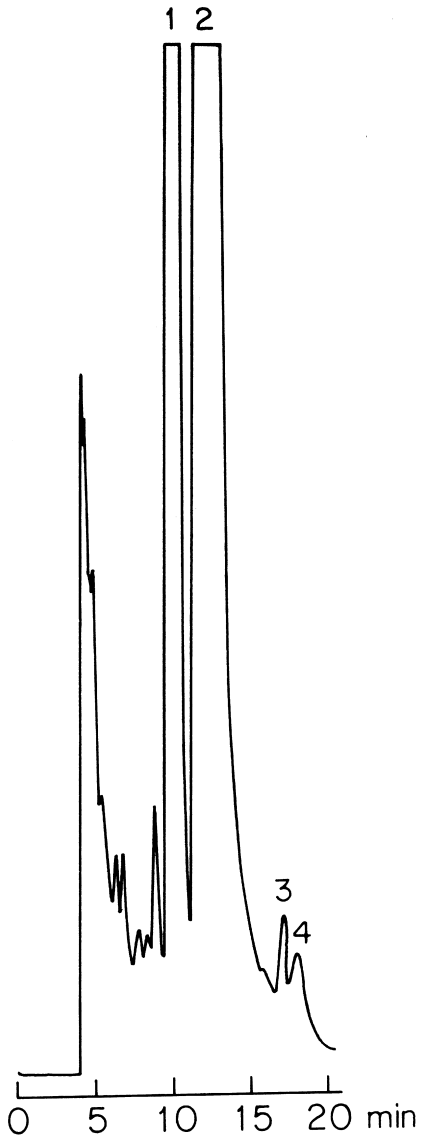

Fig. 4. Chromatogram referring to the analysis of propafenone enantiomers in a plasma sample obtained from a healthy volunteer after administration of (*R*,*S*)-propafenone. (*S*)-Propafenone (3), (*R*)-propafenone (4) and propranonol enantiomers (1,2); Chromatographic conditions were the same as in Fig. 2.

The proposed method proved to be very selective. Among approximately 40 drugs and metabolites (Table 3) evaluated for interference, only amitriptyline, haloperidol, imipramine and diltiazem had elution times close to those of the propafenone enantiomers or to the first peak of propranolol used as

Table 1
Analysis of the precision and accuracy of the method for analysis of propafenone enantiomers in plasma

	(*S*)-Propafenone			(*R*)-Propafenone		
Within-day precision						
Concentration (ng/ml)	25.0	207.8	1995.5	25.0	203.2	1921.7
n	4	10	10	4	10	10
C.V. (%)	6.6	5.9	3.2	8.7	5.2	2.9
Between-day precision						
Concentration (ng/ml)		216.0	1946.9		211.8	1878.7
n		5	5		5	5
C.V. (%)		8.2	6.3		5.5	6.5
Accuracy						
Within-day (%)		3.9	−0.2		1.6	−3.9
Between-day (%)		8.0	−2.6		5.9	−6.1

n, number of determinations; C.V., coefficient of variation.

Table 2
Analysis of the precision and accuracy of the method for analysis of a sample spiked with 100 ng/ml of (*S*)-propafenone and 50 ng/ml of (*R*)-propafenone

	Concentration (ng/ml)		*n*	Precision (C.V.%)	Accuracy (%)
	Real	Obtained			
(*S*)-Propafenone	98.6	103.5	5	3.1	5.0
(*R*)-Propafenone	54.0	59.9	4	6.7	10.8

n, number of determinations; C.V., coefficient of variation.

Table 3
Drugs studied as possible interferents for the determination of propafenone enantiomers in plasma

Drug	t_R (min)	Concentration (μg/ml)	Drug	t_R (min)	Concentration (μg/ml)
(*S*)-Propafenone	18.6	1.25	(*R*,*S*)-Haloperidol	19.5	0.05
(*R*)-Propafenone	19.8	1.25	Imipramine	17.0	0.25
(*R*,*S*)-Propranolol	10.3/12.7	2.50	Lidocaine	6.6	5.00
(*R*,*S*)-5-Hydroxypropafenone	8.6	1.25	(*R*,*S*)-Lorazepam	13.0/22.9	0.24
(*R*,*S*)-Nor-propafenone	8.3	1.25	Mebendazol	16.3	0.12
Albendazole	ND	1.00	(*R*,*S*)-Metoprolol	5.6	0.20
Alprazolam	ND	0.02	(*R*,*S*)-Mexiletine	ND	2.00
Amitriptyline	18.8	0.25	(*R*,*S*)-Oxyphenbutazone	13.0/15.1	95.0
(*R*,*S*)-Atenolol	4.5	0.40	Paracetamol	ND	20.00
Bromazepam	15.7	0.17	Phenobarbital	ND	30.00
Carbamazepine	12.1	12.00	Phenytoin	24.4	20.00
Cimetidine	ND	1.50	(*R*,*S*)-Pindolol	ND	0.02
Clobazam	25.1	0.30	(*R*,*S*)-Praziquantel	24.5/26.0	0.20
Clonazepam	24.5	0.07	Primidone	ND	12.00
Dexamethasone	29.7	0.10	Procainamide	13.5	20.00
Diazepam	38.2	2.50	(*S*,2*R*)-Propoxyphene	5.0	0.75
Diclofenac	24.8	2.00	(*R*,*S*)-Salbutamol	ND	0.20
(*R*,*S*)-Diltiazem	9.3	0.20	Salicylic acid	4.5	170
(*R*,*S*)-Disopiramide	ND	5.00	Triazolam	13.8	0.02
(*R*,*S*)-Fenfluramine	ND	0.15	Trimethoprim	5.2	9.50
Flunitrazepam	32.0	0.02	(*R*,*S*)-Verapamil	13.1/15.6	0.20
Flurazepam	12.2	0.03	(*R*,*S*)-Warfarin	27.5/ 22.9	3.00

t_R, retention time; ND, not detected.

internal standard. This interference could not be avoided by the extraction procedure.

4. Conclusion

In this paper we describe a stereoselective method for the determination of propafenone enantiomers in plasma. Using a solid-phase extraction procedure and a Tris–3,5-dimethylphenyl carbamate cellulose derivative under reversed-phase conditions it was possible to obtain a highly selective method sensitive enough for use in pharmacokinetic studies of propafenone in humans.

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