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Simultaneous determination of propafenone and 5-hydroxypropafenone enantiomers in plasma by chromatography on an amylose derived chiral stationary phase

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Abstract

An enantioselective liquid chromatography method was developed for the simultaneous determination of propafenone (PPF) and 5-hydroxypropafenone (PPF-5OH) enantiomers in plasma. After liquid–liquid extraction with dichloromethane, the enantiomers were resolved on a Chiralpak AD column using hexane-ethanol (88:12, v/v) plus 0.1% diethylamine as the mobile phase and monitored at 315 nm. Under these conditions the enantiomeric fractions of the drug and of its metabolite were analysed within 20 min. The extraction procedure resulted in absolute recoveries of 62.9 and 61.3% for (*R*)- and (*S*)-PPF, respectively, and of 57.6 and 56.5% for (*R*)- and (*S*)-PPF-5OH, respectively. This procedure was efficient in removing endogenous interferents as well the interference of an other PPF metabolite, *N*-despropylpropafenone (PPF-NOR). The calibration curves were linear over the concentration range 25–1250 ng/ml. Low values of the coefficients of variation were demonstrated for both within-day and between day assays. The method described in this paper allows the determination of PPF and PPF-5OH enantiomers at plasma levels as low as 25 ng/ml and can be used in clinical pharmacokinetic studies. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Enantioselective LC analysis; Propafenone; 5-Hydroxypropafenone; Amylose derived chiral stationary phase

1. Introduction

Propafenone (PPF) is an antiarrhythmic drug that is clinically used as a racemic mixture. The

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enantiomers have almost the same activity in their sodium channel-blocking activity, but (*S*)-PPF is 100 times more potent at the β-adrenergic receptors. PPF undergoes extensive first-pass metabolism into two active metabolites: 5-hydroxypropafenone (PPF-5OH) and *N*-despropylpropafenone (PPF-NOR) (Fig. 1). Although they have antiarrhythmic activity comparable to that of PPF, their β-adrenergic activity is negligible [1–5].

In addition to differing in pharmacological activity, PPF enantiomers also differ in their pharmacokinetic properties. Recent reports have demonstrated that (*R*)-PPF inhibits the metabolism of (*S*)-PPF leading to the accumulation of this enantiomer which is responsible for the β-blocking effects. Therefore the disposition of the individual isomers in humans is of clinical relevance [3,6–8].

In order to study the stereoselective disposition of PPF, some chromatographic methods have been reported in literature for the determination of (*S*)- and (*R*)-enantiomers in plasma. Although this resolution can be achieved using chiral derivatization reagents [3,9] or chiral mobile phases [10,11], more direct results have been obtained using chiral stationary phases based on tris(3,5-dimethylphenyl carbamate) derivatives of cellulose and amylose. Hollenhorst and Blaschke [12] reported the resolution of PPF, PPF-5OH and PPF-NOR enantiomers on Chiralcel OD and Chiralpak AD columns. Resolution were obtained for all the compounds by changing the column and the mobile phase composition. Furthermore, the simultaneous resolution of the drug and of PPF-NOR was obtained by using the Chiralcel OD and the Chiralpak AD columns directly connected together. The authors also reported the resolution of PPF enantiomers in a plasma sample using the Chiralpak AD column, but this method was not validated. Aboul-Enein and Bakr obtained lower enantioselectivity in the resolution of PPF [13] employing the Chiralcel OD column. The first completely validated method for the determination of PPF enantiomers in plasma based on chiral stationary phases was reported by Bohm et al. [14], who employed the Chiralpak AD column and hexane-2-propanol-diethylamine as the mobile phase. Recently, the Chiralcel OD-R column was employed under reversed phase conditions to analyse the PPF enantiomers in plasma, after solid phase extraction [15]. Although the chiral resolution of PPF-5OH have already been reported on Chiralcel OD column [12], no method based on chiral stationary phases has been described in the literature for the determination of the enantiomers in plasma samples.

In a recent report [16], we studied the influence of mobile phase composition on the resolution of *rac*-PPF, *rac*-PPF-5OH and *rac*-PPF-NOR using

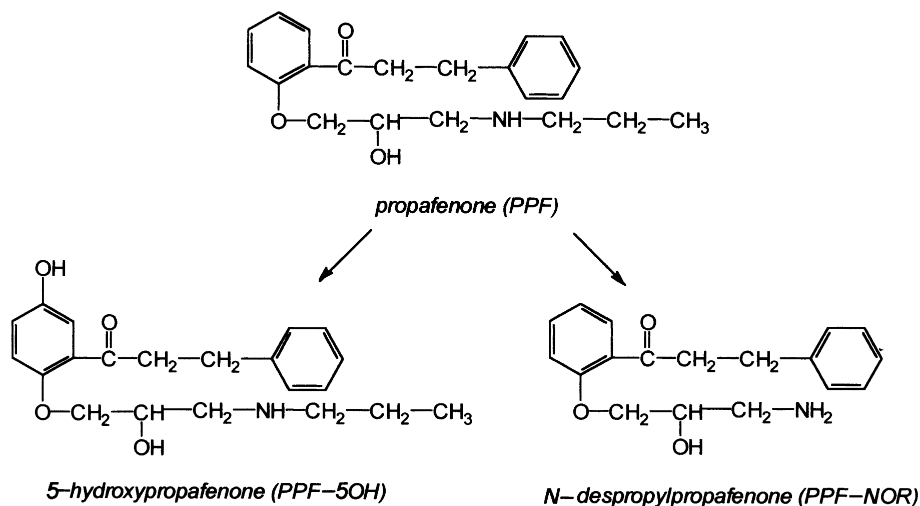

Fig. 1. Metabolism of propafenone.

stationary phases based on proteins or polysaccharide derivatives as the chiral selectors. It was shown that the nature of the alcohol in the mobile phase had a pronounced effect on the resolution of PPF-5OH on the Chiralpak AD column. Thus the enantiomers of PPF and of PPF-5OH could be resolved in a single run, using hexane/ethanol as the mobile phase.

Based on this, we developed an enantioselective LC method for the simultaneous determination of PPF and PPF-5OH enantiomers in plasma. Furthermore, the confidence limits of the method were established for its application to pharmacokinetic studies.

2. Experimental

2.1. Chemicals

PPF hydrochloride and PPF-5OH hydrochloride were kindly supplied by Knoll S.A., Indústrias Químicas (Rio de Janeiro, Brazil) and Knoll A.G. (Ludwigshafen/Rhein, Germany), respectively. Stock solutions containing 1 mg/ml as the base of *rac*-PPF and *rac*-PPF-5OH were prepared in methanol acidified with hydrochloride acid (0.1 mol/l). Working solutions of both compounds (2.0–100.0 μg/ml) were prepared by appropriate dilution in methanol/HCl (0.1 mol/l).

Hexane, dichloromethane (EM Science, Gibbstown, NJ) and ethanol (Merck, Darmstadt, Germany) were chromatography grade. Ammonium chloride, ammonium hydroxide (Merck, Darmstadt, Germany) and diethylamine (Carlo Erba, Milan, Italy) were analytical-reagent grade and were used without further purification.

2.2. Instruments and chromatographic conditions

The HPLC system consisted of an LC10AS solvent pump, an SPD 10A spectrophotometric detector set at 315 nm (0.004 a.u.f.s.), a CR6-A integrator (all from Shimadzu Instruments, Kyoto, Japan) and a 7125 Rheodyne injector with a 50 μl loop (Rheodyne, Cotati, CA). Separations were carried out at room temperature on a Chiralpak AD column (250 × 4.6 mm I.D., 10 μm particle size) purchased from Chiral Technologies, Exton, USA. An RP-8 guard column (4 × 4 mm I.D., Merck, Darmstadt, Germany) was used to protect the analytical column. The mobile phase consisted of hexane-ethanol (88:12, v/v) plus 0.1% diethylamine and the flow rate was 1.3 ml/min with a column inlet pressure of 25 kg/cm^2.

2.3. Extraction procedure

The extraction of PPF and PPF-5OH from human plasma was carried out according to the method of Bohm et al. [14] slightly modified. Plasma samples of 1 ml were transferred to 15 ml glass tubes and alkalinised with 200 μl of buffer solution (NH_4OH/NH_4Cl, pH 11, 0.1 M). After the addition of 3 ml dichloromethane, the tubes were capped, shaken horizontally for 20 min and then centrifuged for 5 min at $1800 \times g$. The organic phases were transferred to clean tubes for another extraction step with 200 μl of the same buffer solution. The organic phases obtained (2 ml) were transferred to clean centrifuge tubes and the solvent was evaporated under a nitrogen stream. The tubes were removed promptly when dried. The residues were dissolved in 100 μl mobile phase and 50 μl was chromatographed. The calibration curves were prepared by adding 25 μl of the working solutions to 1 ml of drug-free plasma in order to obtain the concentration range of 25–500 ng/ml of each enantiomer. The samples were then assayed by the described procedure.

2.4. Recovery and linearity

The analytical recovery of PPF and PPF-5OH enantiomers was determined at concentrations of 25, 125, 250 and 500 ng/ml of each enantiomer ($n = 3$). Drug-free plasma was spiked with known amounts of the drug and metabolite to achieve the concentration previously specified. These samples were submitted to extraction procedure and peak heights were compared with the peak height obtained by the direct injection of the drugs in the mobile phase.

The linearity study was carried out in the range of 25–1250 ng/ml of each enantiomer.

2.5. Precision and accuracy

Detailed precision and accuracy data were obtained by analysing aliquots of three spiked plasma samples at low (50 ng/ml), medium (200 ng/ml) and high (1000 ng/ml) concentration levels of each enantiomer. Within-day reproducibility was determined by analysing ten aliquots of spiked human plasma and between-day reproducibility was determined over a 1-week period ($n=5$).

2.6. Selectivity

5-Hydroxylation reaction is the main pathway for biotransformation of PPF in man, but *N*-dealkylation also occurs. In order to evaluate this interference, PPF-NOR was analysed under the established chromatographic conditions. Interference of other commonly used drugs was also evaluated by injecting solutions of the drugs prepared in the mobile phase onto the chromatographic system and recording their retention times. When the retention time obtained was similar to the retention times of PPF or PPF-5OH enantiomers, a plasma sample spiked with the drug was submitted to the extraction procedure and chromatographic analysis.

2.7. Preliminary human experiment

In order to evaluate the applicability of the method, a plasma sample collected from a healthy volunteer two hours after administration of a single dose of *rac*-PPF (Ritmonor, 150 mg) was analysed under the conditions established in the present study. A blood sample was collected into a heparinized tube and centrifuged at $1800\times g$ for 10 min and the plasma was transferred to a clean tube and stored at -20°C until analysis.

3. Results and discussion

Chromatographic resolution of *rac*-PPF has been obtained using both cellulose and amylose tris(3,5-dimethylphenylcarbamate) derived chiral stationary phases [12–16]. Although, Hollenhorst and Blaschke [12] reported the resolution of *rac*-PPF-5OH on the Chiralcel OD column using hexane-2-butanol (80:20, v/v) acidified with 0.85% acetic acid, no method based on the use of chiral stationary phases has been described in literature for the determination of PPF-5OH enantiomers in plasma. Recently [16], we evaluated the Chiralpak AD column for the enantioselective resolution of PPF and of its metabolites. This column resulted efficient for the resolution of PPF and PPF-5OH enantiomers using hexane/ethanol as the mobile phase. Therefore this column was employed in the development of a new method for the simultaneous determination of the drug and of its main metabolite in plasma. The elution orders on the Chiralpak AD column of both PPF [12,14,16] and PPF-5OH [16] have been previously established.

The extraction procedure at pH 11 employing dichloromethane was based on the method reported by Bohm et al. [14]. When a single-step extraction was used, an interfering peak having the same retention time as that of (*S*)-PPF-5OH was observed in different samples of drug-free human plasma. Its height was found to be subject-dependent. This interfering compound could be eliminated after a washing step of the organic phase with the buffer solution. The chromatograms corresponding to the extract of a 1 ml sample of blank human plasma or of plasma spiked with PPF and PPF-5OH are shown in Fig. 2.

The extraction recoveries reported in Table 1 were determined by comparing the peak height of the extracts with those obtained by direct injection of the same amount of the drug and metabolite. The washing step of the organic phase introduced in the extraction procedure resulted in lower recoveries than that reported by Bohm et al. [14]. Similar recoveries were obtained when plasma samples spiked with the pure enantiomers were submitted to the extraction procedure and chromatographic analysis. In addition, no racemization were observed by analysing these samples.

The calibration curves used in the determination of the linearity of the method were established as described above and their equations were calculated by least-square linear regression. The linear range obtained as well as the typical values

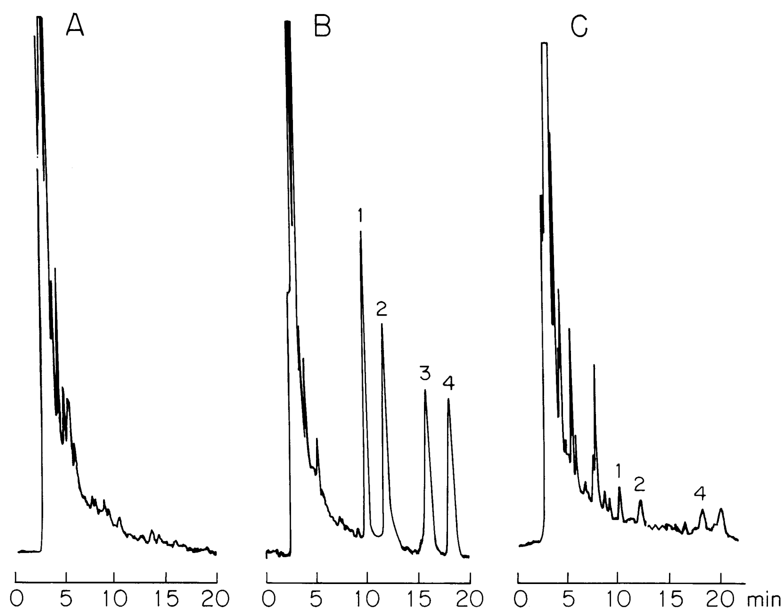

Fig. 2. Chromatograms corresponding to the extract of (A) drug-free plasma, (B) plasma spiked with 250 ng/ml of (*R*)-PPF (1), (*S*)-PPF (4), (*R*)-PPF-5OH (2), and (*S*)-PPF-5OH (3). (C) plasma sample collected after administration of *rac*-PPF.

for the slope, intercept and correlation coefficient are also reported in Table 1.

The method was found to be reproducible, as indicated by the low values obtained for the coefficients of variation (Table 2). The retention times of selected drugs checked for potential interference and which could be administered with PPF are listed in Table 3. Some benzodiazepinic drugs (diazepam, lorazepam, clonazepam and bromazepam) and cimetidine indeed interfere and

Table 1
Recovery and linearity of the method for the determination of PPF and PPF-5OH enantiomers in plasma

	PPF		PPF-5OH	
	(*R*)	(*S*)	(*R*)	(*S*)
Recovery (%)(mean ± S.D.)				
25.0 ng/ml	66.3 ± 4.7	60.9 ± 5.1	59.0 ± 2.9	57.1 ± 3.5
125.0 ng/ml	60.5 ± 3.8	59.6 ± 4.5	54.8 ± 2.7	53.5 ± 3.2
250.0 ng/ml	60.2 ± 3.9	57.3 ± 4.1	59.0 ± 2.7	57.1 ± 3.0
500.0 ng/ml	64.7 ± 3.5	67.4 ± 3.9	57.7 ± 2.7	58.3 ± 2.7
Linearity				
Concentration range (ng/ml)	25–1250	25–1250	25–1250	25–1250
Slope	2.13	1.08	1.22	0.96
Intercept	1.13	3.41	4.54	2.27
Correlation coefficient	0.998	0.998	0.998	0.999

Table 2
Analytical precision and accuracy of the determination of PPF and PPF-5OH enantiomers from spiked plasma samples[a]

Concentration added	Within-day			Between-day		
	Concentration obtained (ng/ml)	CV (%)	Relative error (%)	Concentration obtained (ng/ml)	C.V. (%)	Relative error (%)
50 ng/ml						
(*R*)-PPF	52.5	5.9	5.0	52.3	5.4	4.6
(*S*)-PPF	52.2	6.4	5.0	50.9	9.6	1.8
(*R*)-PPF-5OH	47.6	8.5	−4.4	50.1	8.2	0.2
(*S*)-PPF-5OH	49.4	7.3	−1.2	48.9	8.5	−2.2
200 ng/ml						
(*R*)-PPF	198.4	7.5	−0.8	204.8	6.9	2.4
(*S*)-PPF	198.8	7.0	−0.6	207.7	6.8	3.8
(*R*)-PPF-5OH	195.3	8.2	−2.3	198.6	7.5	−0.7
(*S*)-PPF-5OH	197.1	5.8	−1.4	205.4	6.4	2.7
1000 ng/ml						
(*R*)-PPF	1032.4	6.5	3.1	964.5	6.1	−3.7
(*S*)-PPF	1046.8	2.5	4.5	972.9	6.0	−2.8
(*R*)-PPF-5OH	1041.4	4.8	3.9	968.8	2.1	−3.2
(*S*)-PPF-5OH	1035.2	6.9	3.4	968.9	5.8	−3.2

[a] $n = 10$ for within-day assays and $n = 5$ for between-day assays; CV, coefficients of variation.

this fact should be considered in the application of the method. This interference could not be eliminated by the extraction procedure. The other PPF metabolite, PPF-NOR, eluted from the Chiralpak AD column with a broader peak interfering with the determination of PPF and PPF-5OH enantiomers. However this metabolite was no longer detected after the extraction procedure.

The quantification limit was established as the lower concentration used in the construction of the calibration curves, i.e. 25 ng/ml for all compounds studied. Similar results for PPF enantiomers were reported by Gaitani et al. [15], using the Chiralcel OD-R column. Lower quantification limits (10 ng/ml) were reported by Bohm et al. [14], but it worth to mention that this method did not allow the determination of PPF-5OH enantiomers.

This method can be used to determine the plasma level of PPF and PPF-5OH enantiomers. Fig. 2c shows the chromatogram obtained in the analysis of a sample collected from a healthy volunteer after administration of a single dose of *rac*-PPF.

4. Conclusion

An enantioselective method was developed for the simultaneous determination of PPF and PPF-5OH enantiomers in plasma. This HPLC method is reliable, rapid, selective and sensitive enough to be used in clinical pharmacokinetic studies of the enantioselective disposition of PPF in humans.

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Table 3
Retention times of some drugs and metabolites tested for interference[a]

Drug	t_R (min)	Drug	t_R (min)
Antiepileptics		*Antidepressives*	
Phenytoin	5.2	Amitriptyline	8.0
Carbamazepine	14.1	Imipramine	3.2
Phenobarbital	ND	*Benzodiazepines*	
Valproic acid	5.0	Diazepam	12.6
Cardiovasculars		Clonazepam	16.7
Propranolol	4.5 and 5.2	Alprazolam	ND
Metoprolol	6.1 and 7.8	Triazolam	5.8
Atenolol	ND	Bromazepam	15.7
Pindolol	5.8	Flunitrazepam	24.5
Verapamil	ND	Flurazepam	25.3
Mexiletine	ND	Lorazepam	18.6
Disopyramide	5.0 and 5.7	*Other*	
Lidocaine	19.8 and 22.3	Cimetidine	12.3 and 13.9
Analgesics		Dexamethasone	ND
Acetylsalicylic acid	ND	Albendazole	13.3
Acetaminopheno	ND	Albendazole sulfoxide	6.2
Propoxyphene	6.3	Mebendazole	6.2
Dipirone	19.2		

[a] t_R, retention time; ND, not detected in 60 min; retention time for (*R*)-PPF = 10.0 min, (*S*)-PPF = 18.0 min, (*R*)-PPF-5OH = 12.0 min and (*S*)-PPF-5OH = 16.2 min.

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