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Direct Enantiomeric High Performance Liquid Chromatographic Separation of Propafenone and its Major Metabolite in Serum on a Cellulose Tris-3,5-dimethylphenyl Carbamate Chiral Stationary Phase

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A direct, isocratic and simple liquid chromatographic method is described for the enantiomeric separation of propafenone (PRO) and its major metabolite, 5-hydroxypropafenone, using a cellulose tris-3,5-dimethylphenyl carbamate (Chiralcel ODS) column. The stereochemical separation factor (α) obtained was 1.14 and the maximum stereochemical resolution factor (*R*) was 0.80 when using a mobile phase consisting of hexane: 2-propanol: diethylamine (90:10:0.4) at 23 °C. The hydroxy metabolite could not be successfully separated into its corresponding enantiomers under these chromatographic conditions. The method has been used to determine and identify the enantiomers of PRO and its hydroxy metabolite in a serum sample.

INTRODUCTION

Propafenone (PRO) is a potent class 1c antiarrhythmic agent with moderate β-blocking activity and weak calcium channel blocking actions that is effective against supraventicular and ventricular arrhythmias (Dukes and Vaughan-Williams, 1984; Singh, 1987; Smith *et al.*, 1987; Harron and Brogden, 1987).

Currently, PRO (Fig. 1) is administered as a racemic mixture consiting of 50% (−)-*R* enantiomer and 50% of (+)-*S* enantiomer. These two isomers possess different β-blocking properties but have equal effects on the sodium channel-dependent antiarrhythmic class 1 activity (Burnett *et al.*, 1988; Kroemer *et al.*, 1989; Stoschitzky *et al.*, 1990).

Kroemer *et al.* (1989) concluded that the administration of optically pure (*R*)-PRO might be of advantage in patients intolerant to β-blockade, although their study lacks sufficient evidence of the β-blocking effects of (*S*)-PRO in humans. Recently, Stoschitzky *et al.* (1990) substantiated the concept of administering optically pure (*R*)-PRO in order to achieve more specific antiarrhythmic class 1c therapy with less β-blocking side-effects instead of the currently used racemic mixture. However, when additional β-blockade is needed, it could be attained by co-administration of such pure β-adrenoceptor antagonists such as atenolol or propranolol. For this reason, resolution of racemic PRO to its corresponding enantiomers on a large scale by chemical methods has been reported (Lindner, 1987).

PRO undergoes extensive metabolism in the liver hydroxylation is the main metabolic route with the production of 5-hydroxypropafenone (5-HPRO, Fig. 1) (Hege *et al.*, 1984; Kates *et al.*, 1985), a metabolie with antiarrhythmic properties *in vivo* (Valenzuela *et al.*, 1987; Von Philisborn *et al.*, 1984).

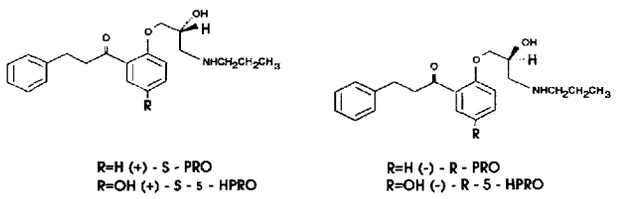

Figure 1. The absolute configurations of PRO and 5-HPRO.

Fundamental to studies of stereoselective differences in pharmacodynamics and pharmacokinetics are analytical methods for the determination of enantiomeric purity of composition. Chromatographic enantioseparation is becoming the method of choice for the determination of the enantiomeric composition of drugs in biological fluids and also in bulk samples.

Mehvar (1990) reported the separation of PRO enantiomers in human plasma samples after derivatization with (−)-*R*-naphthylethyl isocyanate. Gal *et al.* (1990) also reported a method for the resolution of PRO enantiomers via derivatization with homochiral derivatizing agents, namely 2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl isothiocyanate and (*S*)-(1-naphthyl)ethyl isothiocyanate.

Described here is a direct and simple high performance liquid chromatographic (HPLC) method for the enantiomeric separation of the enantiomers and the 5-hydroxy metabolite (5-HPRO) of PRO on the commercially available cellulose tris-3,5-dimethylphenyl carbamate chiral stationary phase on silica gel (Chiralcel OD). The method could be applied for preparative scale chromatogrpahy for resolution of racemic PRO to its individual enantiomers. Furthermore, the method could also be used to separate and analyse PRO enantiomers and 5-HPRO in biological fluids.

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EXPERIMENTAL

Apparatus. The Waters Liquid Chromatography System (Waters Associates, Milford, MA, USA) consisted of a Model M-45 pump, a U6K injector and a Lambda-Max Model 481 LC spectrophotometer UV detector operated at 247 nm. The stationary phase of the Chiralcel OD analytical column of cellulose tris-3,5-dimethylphenyl carbamate (25 × 0.46 cm, i.d.; Daicel Chemical Industries, Tokyo, Japan) coated on silica gel with particle size 10 μm was used.

Chemicals. Racemic PRO and its corresponding (+)-*S* enantiomer were kindly supplied by Professor W. Lindner, Institut Für Pharmazeutishche Chemie, Karl-Franzene Universität, Graz, Austria. 5-HPRO (Lot No. LU40545) was obtained from Knoll, AG (Ludwigshafen, Germany). HPLC-grade hexane and 2-propanol, and diethylamine were obtained from Fisher Scientific, Fairlawn, NJ, USA.

Chromatographic conditions. The maximum and symmetrical stereochemical resolution of PRO was obtained using hexane and 2-propanol (90:10) containing 0.4% (v/v) diethylamine on a Chiralcel OD column. The flow-rate was 1.0 mL/min and the chart speed was 0.5 cm/min. A temperature of 23 °C and a pressure of 300 psi were maintained throughout the experiment. Detection was obtained at UV 247 nm with a sensitivity range of 0.01 aufs. The sample amount injected was 3.0 nmol for racemic PRO and 1.5 nmol for the (+)-*S* enantiomer.

Sample pretreatment. Racemic propafenone hydrochloride (100 mg) and 5-hydroxypropafenone hydrochloride (50 mg) was added to 10 mL human serum. The serum was diluted up to 150 mL with water, basified with sodium biocarbonate and extracted with dichloromethane (3 × 25 mL). The combined dichloromethane extract was dried over sodium sulphate and filtered and the solvent was removed under vacuum. The residue was again acidified using methanolic hydrogen chloride solution, dried under a stream of nitrogen. The residue was dissolved in methanol and injected onto the column.

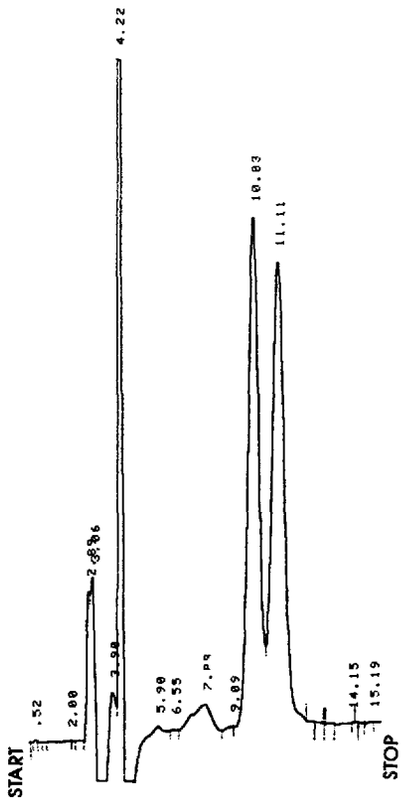

Figure 2. Enantiomeric separation of racemic PRO. Column: Chiralcel OD (250 × 4.6 mm, i.d.); mobile phase: hexane:2-propanol:diethylamine (90:10:0.4 v/v); flow-rate: 1.0 mL/min; chart speed: 0.5 cm/min; temperature: 23 °C; detector: UV 247 nm; sensitivity: 0.01 aufs; sample quantity: 3 nmol.

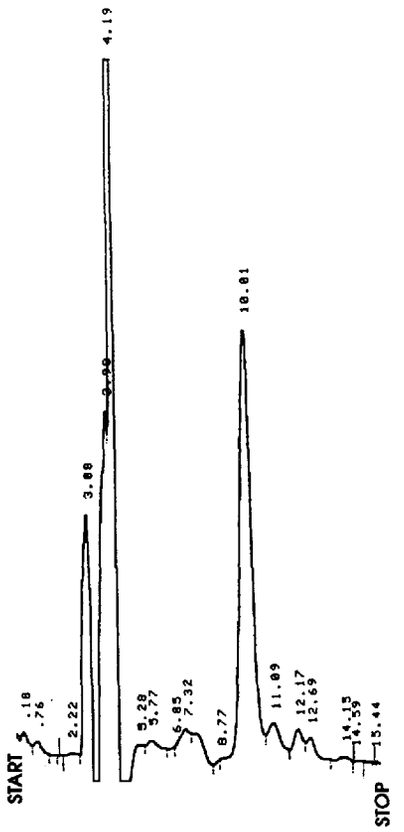

Figure 3. Chromatogram of (+)-*S*-PRO. Conditions were the same as in Fig. 2, except that the quantity of sample injected was 1.5 nmol.

Determination of enantiomeric elution order. The enantiomeric elution was determined by chromatographing (+)-*S*-PRO separately under the same conditions. Thus, the peak that eluted with the lower capacity factor was identified as (+)-*S*-PRO and the one that eluted with the higher capacity factor was identified as (−)-(*R*)-PRO.

RESULTS AND DISCUSSION

The method reported here is one where PRO enantiomers can be directly resolved using cellulose tris-3,5-dimethylphenyl carbamate (Chiralcel OD) without derivatization as in the previously reported methods by

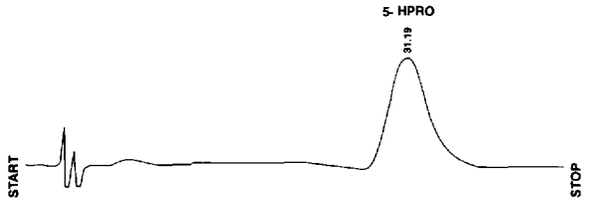

Figure 4. Chromatogram of the racemic 5-HPRO metabolite. Conditions were the same as in Fig. 2, except that the quantity of sample injected was 3 nmol.

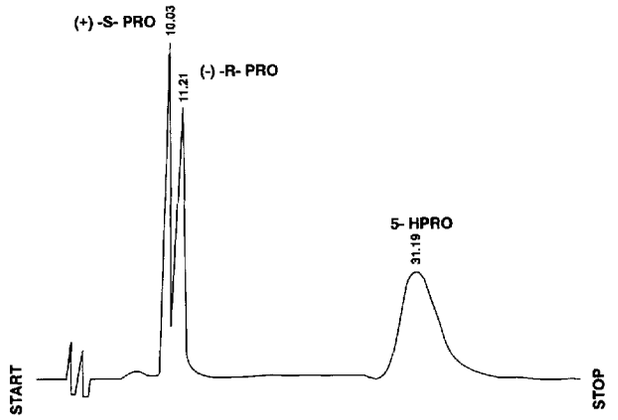

Figure 5. Chromatogram obtained from the methylene chloride extract of 10 mL of human serum to which racemic PRO and 5-HPRO were added. Chromatographic conditions were the same as in Fig. 1.

Mehvar (1990) and Gal *et al.* (1990). The method is simple and fast as it only requires about 15 min for the resolution of the PRO enantiomers.

This cellulose-derived chiral stationary phase has been successfully used for the direct separation of several β-adrenergic blockers of the aryloxyaminopropanol group, such as penbutolol, celiprolol, carazolol, timolol (Aboul-Enein and Islam, 1989, 1990a, b, c), among others (Okamoto *et al.*, 1986), into their respective enantiomers. Because of the structural similarity between PRO and these β-adrenergic blockers, since both possess an asymmetric carbon atom in the aminopropanol chain that is common to most β-adrenoceptor antagonists, it was decided to use the Chiralcel OD column to effect the direct resolution of PRO enantiomers.

Different concentrations of 2-propanol and diethylamine in hexane were used as a mobile phase to optimize the separation of PRO enantiomers and its metabolite. A typical chromatogram of the enantioseparation of PRO is shown in Fig. 2. By comparison with the chromatogram and the capacity factor of (+)-*S*-PRO (Fig. 3), the peak which eluted at a lower capacity factor ($k_1 = 2.57$) was identified as the (+)-*S* enantiomer and the peak with the higher capacity factor ($k_2 = 2.93$) as the (−)-*R* enantiomer. The stereochemical separation factor (α) was 1.14. The maximum stereochemical resolution factor (*R*) was 0.80. However, the 5-HPRO metabolite could not be resolved to its corresponding enantiomers under the same chromatographic conditions. The metabolite was eluted as a single peak at $k = 9.40$ (Fig. 4).

The enantiomeric separation of the dichloromethane extract of racemic PRO and 5-HPRO in a human serum sample is shown in Fig. 5. All the peaks were easily identified from retention times and capacity factors by comparison with Figs. 2 and 4.

CONCLUSION

The direct stereochemical separation of racemic PRO was achieved without derivatization on commercially available cellulose 3,5-dimethylphenyl carbamate under isocratic conditions. The method was applied to the determination and identification of the enantiomers of PRO and its major-hydroxy metabolite in serum.

This method could be used for the optical purity determination of PRO in bulk and in pharmaceutical formulations. Furthermore, the method could be applied on a preparative scale for the separation of a large quantity of PRO enantiomers by using the preparative Chiralcel OD column which is commercially available. This method has the advantage of being simple and fast and can be adopted to quantitate the enantiomers of PRO and its 5-hydroxy metabolite in biological fluids for further pharmacodynamic and pharmacokinetic studies.

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