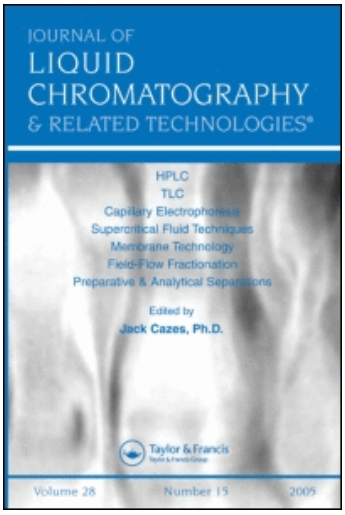

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Separation and Determination of Propranolol Enantiomers in Plasma by High-Performance Liquid Chromatography with Fluorescence Detection

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SEPARATION AND DETERMINATION OF PROPRANOLOL ENANTIOMERS IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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ABSTRACT

The separation and determination of (-)- and (+)-propranolols in plasma by high-performance liquid chromatography (HPLC) are described. The newly developed method is based on precolumn derivatization with a chiral axis reagent, (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide (MBNCC), to form diastereomeric esters. The diastereomers were separated on a normal phase column by HPLC with fluorescence detection employing 0.3% methanol in hexane-ethyl acetate (15:1, v/v) as the mobile phase. The clean-up of propranolol in plasma was efficiently attained by the combined use of a Sep-pak C_{18} cartridge and an ion exchange gel, carboxymethyl Sephadex LH-20. The detection limit of each propranolol enantiomer was 100 pg.

INTRODUCTION

A reliable method for the simultaneous determination of each enantiomeric drug in biological fluids is a prerequisite for pharmacokinetic studies of the racemate. Among various

methods, high-performance liquid chromatography (HPLC) has been recognized as a powerful tool for the resolution of enantiomers. The diastereomeric method, employing a chiral derivatization reagent, is more favorable for the determination of enantiomeric drugs in biological specimens with respect to sensitivity and versatility. In the previous papers, we reported the preparation of chiral derivatization reagents for the resolution of enantiomeric amines [1] and carboxylic acids [2,3], and their use for the separation and determination of enantiomeric drugs in biological fluids by HPLC [4,5]. We also synthesized new chiral fluorescence derivatization reagents, (-)- and (+)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanides (MBNCC), having carbonyl cyanide as a reacting group toward the hydroxyl function, and demonstrated their applicability to the resolution of enantiomeric hydroxyl compounds by HPLC [6]. This paper deals with the use of the precolumn derivatization reagent for the separation and determination of propranolol enantiomers in plasma by HPLC on a normal phase column.

EXPERIMENTAL

Materials

The derivatization reagent, (-)-MBNCC, was synthesized in the manner previously reported [6]. Bufuralol enantiomers were kindly donated by Roche Products Ltd. (Welwyn Carden, England). Carboxymethyl Sephadex LH-20 (CM-LH-20)(1 mequiv./g) was prepared according to the known method [7]. The Sep-pak C_{18} cartridge was purchased from Millipore-Waters (Milford, MA, U.S.A.) and washed successively with ethanol, 5% aqueous bovine serum albumin solution and then water prior to use. All other chemicals employed were of analytical reagent grade. Solvents were purified by distillation prior to use. All glassware used was silanized with trimethylchlorosilane.

Instruments

The apparatus used for this work was a M-45 solvent delivery system (Millipore-Waters) equipped with a 650-10LC fluorescence spectrophotometer (excitation wavelength 318 nm; emission wavelength 408 nm)(Hitachi, Tokyo). A Spherical Silica (5 μm, 15 cm x 4.6 mm I.D.)(Millipore-Waters) column was used at ambient temperature.

Procedure for the separation and determination of propranolol enantiomers in plasma

To a plasma specimen (1 ml) was added (+)-bufuralol (10 ng) as an internal standard (IS), and the whole was diluted with 0.5M sodium phosphate buffer (pH 7.0, 4 ml) and passed through a Sep-pak C_{18} cartridge. After washing with water (5 ml) and 30% ethanol (5 ml), β-blockers were eluted with ethanol (8 ml). The eluate was evaporated down, mixed with (-)-MBNCC (200 μg) in 0.01% quinuclidine in acetonitrile (100 μl), and the resulting solution was heated at 60°C for 20 min. The excess reagent was decomposed with methanol (50 μl) and the mixture was evaporated down. The residue obtained was redissolved in 90% ethanol (1 ml) and applied to a column (18 mm x 6 mm I.D.) of CM-LH-20 (100 mg). Elution was carried out at a flow rate of 0.2 ml/min. After washing with 90% ethanol to remove neutral and acidic compounds, diastereomers formed were eluted with 0.1M methylamine in 90% ethanol (3 ml). The dried eluate was redissolved in the mobile phase (50-100 μl) and a 10-20 μl aliquot of the solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Derivatization of propranolol enantiomers with (-)-MBNCC into diastereomeric esters

In the previous paper, we demonstrated that β-blockers such as propranolol and penbutolol were easily derivatized into

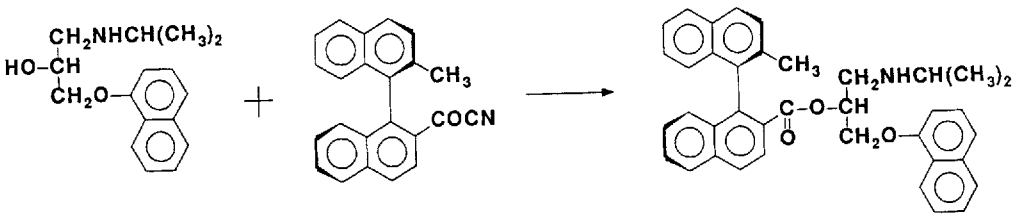

FIGURE 1. Derivatization of propranolol enantiomers with 2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide.

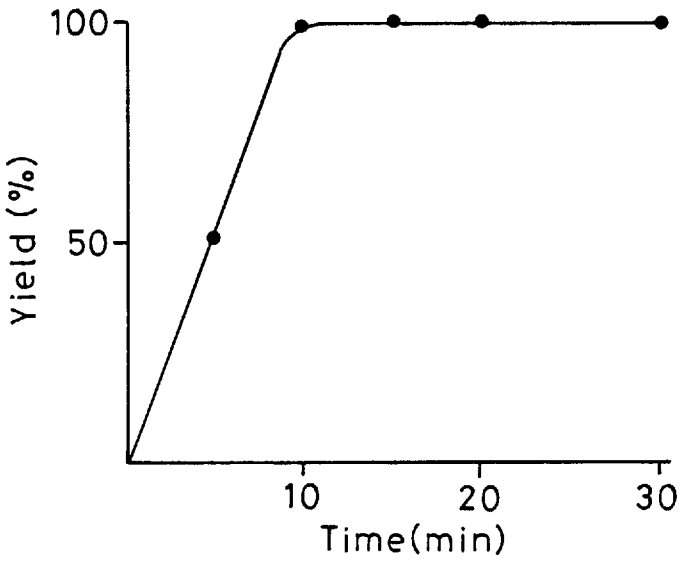

FIGURE 2. Time course for derivatization of propranolol enantiomers with (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide.

diastereomeric esters through the inherent secondary alcoholic group at the chiral center with fluorescence derivatization reagents, (-)- and (+)-MBNCC (Fig. 1)[6]. Therefore, suitable conditions for the condensation of propranolol with the reagent were investigated. Propranolol enantiomers (each 100 ng) and (-)MBNCC (200 μg) were dissolved in 0.01% quinuclidine in acetonitrile (100 μl) and allowed to stand at 60°C. An aliquot of the solution was applied to HPLC and the yields of fluorescent esters were estimated by comparison of the peak areas with those of the standard samples. As shown in Fig. 2, the reaction rate increased along with the reaction time up to 10 min, resulting in a quantitative formation of diastereomeric esters. No racemization of the reagent itself took place during the

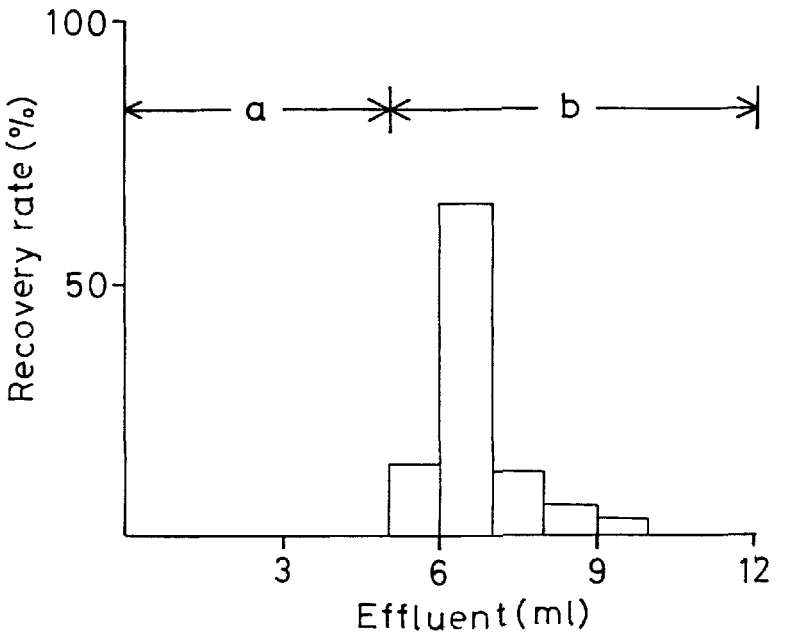

FIGURE 3. Elution pattern of propranolol on a Sep-pak C_{18} cartridge.
Eluent: a) 30% ethanol, b) ethanol.

condensation reaction. On the basis of these data, propranolol enantiomers were treated with (-)-MBNCC in 0.01% quinuclidine in acetonitrile at 60°C for 20 min.

Clean-up of propranolol in plasma

The separation and determination of trace components in biological specimens are markedly influenced by the clean-up procedure employed. The cartridge packed with ODS bonded silica is widely used for extraction of drugs and their metabolites in biological fluids. In the present study, a Sep-pak C_{18} cartridge was used for extraction of propranolol in plasma.

Propranolol in sodium phosphate buffer (pH 7.0) was applied to the cartridge. After washing with water and 30% ethanol to remove co-existing inorganic salts and other polar substances, the eluate obtained with ethanol was then separated and analyzed by HPLC. As illustrated in Fig. 3, the β-blocker was recovered at the rate of more than 90% in an initial 3 ml of the effluent.

Further purification was undertaken employing a lipophilic cation-exchanger, CM-LH-20. After derivatization with (-)-MBNCC, the reaction product was dissolved in 90% ethanol and applied to a column of CM-LH-20. The neutral and acidic interfering compounds were almost entirely removed by eluting with 90% ethanol and the desired basic diastereomers were quantitatively recovered with 0.1M methylamine in 90% ethanol. The efficient clean-up procedure for HPLC of propranolol in plasma was thus established and an excellent chromatogram of the enantiomers was obtained without any interfering peaks (Fig. 4a, b).

Determination of propranolol enantiomers in plasma

The standard procedure for the separation and determination of propranolol enantiomers in human plasma is shown in Fig. 5. After addition of (+)-bufuralol as an IS, a plasma specimen was extracted with a Sep-pak C_{18} cartridge and then treated with (-)-MBNCC in the presence of quinuclidine in acetonitrile according to the procedure described above. After purification with CM-LH-20, fluorescent diastereomers obtained were subjected to HPLC on a Spherical Silica column using 0.3% methanol in hexane-ethyl acetate (15:1, v/v) as a mobile phase and monitored by fluorescence detecction (excitation wavelength 318 nm; emission wavelength 408 nm), the limit of detection being 100 pg. A calibration graph was constructed by plotting the ratio of the peak area of (-)- or (+)-propranolol to that of (+)-bufuralol against the amount of propranolol enantiomers, a

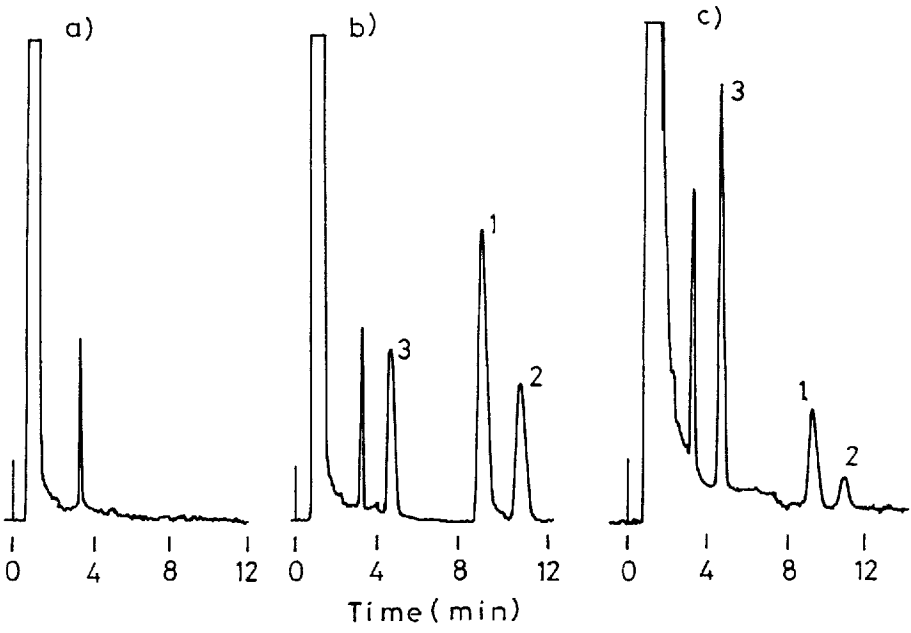

FIGURE 4. Chromatograms of (-)- and (+)-propranolols in human plasma.
a) Control plasma; b) plasma spiked with (-)-propranolol (25 ng/ml), (+)-propranolol (13 ng/ml) and (+)-bufuralol (10 ng/ml); c) plasma obtained 1 hr after oral administration of (±)-propranolol (10 mg). 1=(-)-Propranolol; 2=(+)-propranolol; 3=(+)-bufuralol (IS). Conditions: column, Spherical Silica; mobile phase, 0.3% methanol in hexane-ethyl acetate (15:1, v/v).

linear response to each enantiomer being observed in the range 500 pg-100 ng/ml.

Applying the standard procedure to human plasma, propranolol enantiomers were determined with satisfactory reproducibility. Known amounts of propranolol were added to plasma and their recovery rates were estimated. As listed in Table I, both (-)- and (+)-propranolols were recovered at a rate

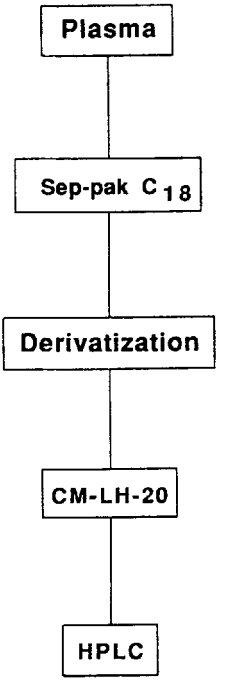

FIGURE 5. Procedure for determination of propranolol enantiomers in human plasma.

TABLE I

Recovery of (-)- and (+)-Propranolols Added to Human Plasma

Enantiomer	Propranolol (ng/ml)		Recovery ± S.D. (%)*
	Added	Found	
(-)-Propranolol	3.1	2.8	90.3 ± 8.2
	10.9	10.4	95.4 ± 5.8
(+)-Propranolol	2.9	2.6	89.7 ± 8.5
	10.2	9.8	96.1 ± 6.2

*n=8

of more than 90%. A typical chromatogram of propranolol enantiomers in human plasma obtained 1 hr after oral administration of (±)-propranolol (10 mg) is illustrated in Fig. 4c. The (-)- and (+)-propranolol peaks on the chromatogram represent approximately 3 and 1 ng/ml, respectively. This result is fairly in agreement with the previous findings [8,9]. The chromatogram with a stable baseline and without leading and tailing is favorable for the determination of propranolol enantiomers in plasma.

It is hoped that the availability of a new method for the simultaneous determination of enantiomeric drugs in biological fluids with satisfactory reliability and sensitivity may provide more precise information on the pharmacokinetics of β-blockers.

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