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Microassay of propranolol enantiomers and conjugates in human plasma and urine by high-performance liquid chromatography after chiral derivatization for pharmacokinetic study

C. PHAM-HUY,*† A. SAHUI-GNASSI,† V. SAADA,† J.P. GRAMOND,‡ H. GALONS,§ S. ELLOUK-ACHARD,† V. LEVRESSE,† D. FOMPEYDIE‡ and J.R. CLAUDE†

† *Laboratoire de Toxicologie (E.A. 207)*
‡ *Laboratoire de Chimie Analytique*
§ *Laboratoire de Chimie Organique, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France*

Abstract: A microdetermination of propranolol enantiomers and of their glucuronide and sulphate conjugates in human plasma and urine by reversed-phase HPLC after chiral derivatization is described. After extraction from 100 μl of plasma or urine with racemic 4-methylpropranolol as internal standard (I.S.), the enantiomers are derivatized with R(+)-phenylethylisocyanate as chiral derivatization reagent. Chromatography is performed on Novapak C_{18} column with fluorescence detection. Glucuronide and sulphate conjugates are cleaved prior to extraction by incubating, respectively, the samples with glucuronidase–arylsulphatase and saccharic acid 1–4 lactone as specific glucuronidase inhibitor. The retention times of propranolol and I.S. enantiomer derivatives are short (t_R = 5.5–6.2 min and 8.8–10.1 min, respectively). The diastereomeric derivatives are very stable and show good peak symmetry and resolutions (R_S = 2 and 2.2). The use of 4-methylpropranolol as I.S. improves significantly relative standard deviations (RSD: 1.7–5.1). Sensitivity is about 1 ng ml^{-1} per enantiomer. The method is applied to pharmacokinetic studies of racemic propranolol in human plasma and urine. S-propranolol and its conjugates show higher concentrations than R-propranolol and its conjugates in plasma and urine.

Keywords: *Propranolol enantiomers, conjugates, 4-methylpropranolol, reversed-phase HPLC, derivatization, pharmacokinetics.*

Introduction

Propranolol, a non-selective β-adrenergic blocking agent, is widely prescribed in the treatment of cardiovascular diseases (hypertension, angina pectoris and cardiac arythmias) and the prophylaxis of secondary acute myocardial infarction [1]. Propranolol used in therapy is a racemic mixture of the (S)-and (R)-enantiomers which have large differences in metabolism, pharmacokinetics and pharmacological activity [2, 3]. (S)-propranolol is about 100 times more potent than the corresponding (R)-isomer upon β-receptors [2]. Therefore, it is interesting to study the measurement of the respective enantiomers in biological fluids rather than the total concentrations in order to know their individual therapeutic profiles and their kinetic interaction.

The previously described methods for the separation of propranolol enantiomers were either immunochemical or physicochemical assays. Only two enantioselective immunoassays were cited in literature; one of two used radioimmunoassay [4], and recently, another method was performed by an enzyme-linked immunosorbent assay (ELISA) [5]. Some other immunochemical assays cannot distinguish between the two enantiomers [6–8]. It is known that the formation of a selective antibody depends on the immunogen synthesis [5]. A weak cross-reactivity of antibody to the other enantiomer was notified in these two enantioselective immunoassays [4, 5].

Enantiospecific physicochemical assays allow the simultaneous measurement of both enantiomers but do not account for active (unbound) species only [9]. These methods include gas chromatography–mass spectrometry using deuterium labelled pseudoracemates [10], radioreceptor assay [11], thin layer chromatography after chiral derivatization [12] and high-performance liquid chromatography

* Author to whom correspondence should be addressed.

(HPLC) using chiral stationary phases such as Pirkle type 1A, i.e. (R)-*N*-(3,5-dinitrobenzoyl)-phenylglycine [13], α-1-acid glycoproteins [14], cyclodextrins [15, 16] and derivatized cellulose [17] or HPLC after chiral derivatization [9, 18–23] or normal-phase HPLC using chiral eluents [24]. Reversed-phase HPLC using optically active derivatization reagents is the most common procedure used in the determination of propranolol enantiomers in man and animal because of the low cost of the column and the high sensitivity.

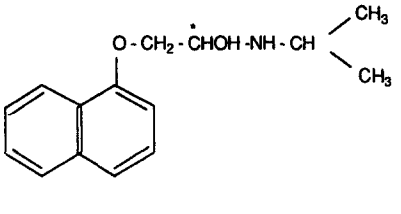
Propranolol is an aminoalcohol compound containing a secondary hydroxy group and a secondary amine function accessible for derivatization (Fig. 1). A number of chiral derivatizing reagents such as R(+)-phenylethylisocyanate (PEIC) [9, 18, 19], 2,3,4,6 tetra-O-acetyl β-D-glucopyranosyl isothiocyanate (TAGIT) [20], S-flunoxaprofen isocyanate (FLOPIC) [9], S-flunoxaprofen chloride (FLOP-Cl) [9], S(+)-benoxaprofen chloride [12], (−) menthyl chloroformate [21], tert-butoxycarbonyl-L-leucine anhydride [22] and (R,R)-O,O-diacetyltartaric acid anhydride (DATAAN) [23], have been used to convert propranolol into diastereomeric derivatives by attacking either the amine function or the secondary hydroxy group. PEIC converts the secondary amine of propranolol into the urea diastereomeric derivatives (Fig. 1) which can be separated on a reversed-phase column [25].

The aim of this study is to report an improved microanalysis of propranolol enantiomers and their conjugates in plasma and urine utilizing 4-methylpropranolol (Fig. 1) as an internal standard and reversed-phase HPLC after chiral derivatization with PEIC. This rapid method was applied to the determination of S- and R-propranolol, their sulpho- and glucuro-conjugates in human plasma and urine at different times after oral administration of the racemic drug.

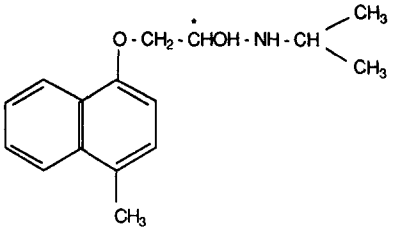
Experimental

Materials

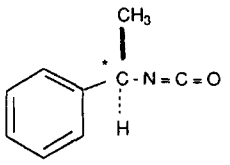
Racemic-, S(−)- and R(+)-propranolol hydrochloride and racemic 4-methyl-propranolol hydrochloride used as internal standard were purchased from Cambridge Research Biochemicals (Gadbrook Park Northwich, Cheshire CW 9-7RA, UK). R(+)-phenylethyl-isocyanate was obtained from Fluka (Buchs, Switzerland), β-glucuronidase–arylsulphatase (purified powder, type H1) from helix pomatia (Sigma, St Louis, USA) and D-saccharic acid 1-4 lactone (Sigma) were used for measuring



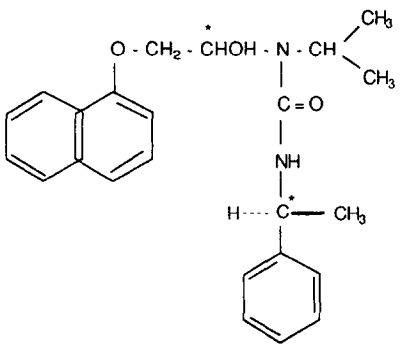
Propranolol



4-Methylpropranolol (Internal Standard)



R-(+)-Phenylethylisocyanate



urea diastereomeric derivative of propranolol

Figure 1
Structures of propranolol, internal standard, chiral derivatizing reagent and diastereomeric derivative.

glucuronide and sulphate conjugates. All solvents were of HPLC grade (Merck, Darmstad, Germany). Other chemicals (Merck) were of analytical purity. Deionized water was purified by Milli Q-UV Plus system (Millipore, Milford, USA). Micro Millex filters Model GV 0.4 μm (Millipore) were used to filter extracted samples.

Instrumentation and chromatographic conditions

The HPLC system consisted of an SP 8810 isocratic pump (Spectra Physics, France), a Shimadzu RF 535 fluorescence detector (Shimadzu, Kyoto, Japan), a Shimadzu C-R6A integrator, and a Rheodyne injector, Model 7125, fitted with 50 μl loop. An analytical column (150 × 3.9 mm i.d.) was packed with Novapak C18 reversed-phase, 4 μm particles and purchased from Waters–Millipore (Milford, USA). A guard column (15 × 3.2 mm i.d.) packed with 7 μm, wide pore, spherical C18 silica (Brownlee Labs, France) was connected between the injector and the analytical column.

The mobile phase was composed of methanol–water (72.5:27.5, v/v), then degassed by sonication and pumped at 1.6 ml min^{-1}. Injection volumes of samples and standards were made with a 50 μl sample loop. Column temperature was ambient (18–21°C). Fluorescence detector was set at 232 nm (excitation) and 340 nm (emission).

Preparation of standards

Four stock standard solutions were prepared by dissolving individually racemic, (S)- and (R)-propranolol hydrochloride and racemic 4-methylpropranolol hydrochloride in methanol at 1.0 mg ml^{-1} (free base). Stock solutions were stable for one month at 4°C. Calibration solutions were prepared by spiking drug-free human plasma or urine with stock standard solution to achieve final concentrations of 20, 50, 100, 200, 500, 1000 ng ml^{-1} racemic propranolol base (i.e. 10, 25, 50, 100, 250, 500 ng ml^{-1} of each enantiomer) and stored at −20°C. An internal standard working solution at 1 μg ml^{-1} was prepared by dilution of an aliquot of racemic 4-methylpropranolol stock solution with methanol.

Extraction and derivatization procedure

Unconjugated propranolol. One hundred μl sample of thawed plasma, urine or standard calibration solution were added to a labelled 11 × 100 mm centrifuge glass tube, then followed successively by 100 μl of internal standard in methanol, 100 μl of concentrated ammonium hydroxide (25%) and 2 ml of methanol–diethylether mixture (10:90 v/v). The tubes were tightly capped and mixed by vortex (Maximix, Bioblock, France) for 1.5 min. After centrifugation for 5 min at 1500***g***, the upper organic layer was transferred into a 5 ml glass tube and evaporated under a stream of nitrogen at room temperature (18–22°C). One hundred μl of diluted PEIC solution in diethylether (5 μl ml^{-1}) were added into each tube of sample extract. The tubes were vortexed (Maximix) for 30 s and kept for 30 min at room temperature. After evaporation under a stream of nitrogen at room temperature, the residue was dissolved by vortex for 30 s with 100 μl of mobile phase. For plasma extract, the reconstituted liquid was centrifuged for 7 min at 3000***g*** or filtered through a micro Millex filter to separate the precipitate. For urine extract, this last operation was unnecessary. A 50 μl aliquot of the extract was then injected into the column. For duplicate injections, all previous volumes were doubled, except for extraction solvent which can be maintained at 2 ml without loss of recovery.

Glucuronide and sulphate conjugates. Each 100 μl of unknown plasma or urine diluted from 1:2 to 1:10 was added into two labelled 11 × 100 mm glass tubes. For the determination of the propranolol glucuronide, the sample was rapidly vortexed with 50 μl of β-glucuronidase–sulphatase solution at 32 mg ml^{-1} in sodium acetate buffer 0.01 M, pH 5, then incubated overnight (14 h) at 37°C. For the determination of the propranolol sulphate, 50 μl of saccharic acid 1-4 lactone (8 mg ml^{-1} in acetate buffer) used as specific inhibitor of β-glucuronidase were added to the sample before the addition of 50 μl β-glucuronidase–sulphatase solution. The second tube was rapidly vortexed and also incubated overnight at 37°C. For duplicate injections, double volumes of samples, enzymes and enzyme inhibitor were used.

After incubation, the two tubes of glucuronide and sulphate conjugates were treated by the same extraction and derivatization procedure as previously described. A blank plasma or urine was incubated with glucuronidase–

arylsulphatase and saccharo-lactone, then treated as above.

Protocol for pharmacokinetic investigation

Four healthy volunteers (three females weighing about 50 kg, one male, 66 kg and aged from 24 to 42 years) orally received a single dose of racemic propranolol in the morning (7.30 h). Two of them were administered with 80 mg of racemic drug; two other subjects were given 40 mg of the same compound. Five ml blood samples were taken in heparin 2, 4 and 8 h after administration. After centrifugation, the plasma was removed and frozen at −20°C before use. At the same time, the urines of 24 h were collected separately. Before administration, all subjects urinated completely and these urines were rejected. After administration of drug, each urine volume was measured and time was noted. Each urine aliquot was also stored at −20°C.

Calculation

The quantification of each propranolol enantiomer in the sample was based on the peak area ratio of S- or R-propranolol to the corresponding enantiomer of the internal standard. This was calculated from a standard calibration curve obtained under the same conditions with standard calibration solutions in the range of studied concentrations. The identification of S- or R-propranolol in racemic mixture was based on the comparison of their retention time with that of S- or R-propranolol standards.

The concentrations of sulphate conjugate enantiomers in the sample were calculated as the difference between the quantity of propranolol enantiomers found in the 'sulphate tube' and the quantity of unconjugated propranolol enantiomers and were given as propranolol enantiomer equivalents. The amounts of glucuronide conjugate enantiomers in the sample were also calculated as the difference between the quantity found in the 'glucuronide tube' and the quantity found in the 'sulphate tube' and were given as above. The concentration of each enantiomer in plasma was expressed in ng ml^{-1} of propranolol base. The concentration of each enantiomer in urine was expressed in ng ml^{-1} and μg total excreted in each urine volume. For the determination of conjugates in urine, the dilution ratio must be taken in the calculation.

Results

Analytical variables

Chromatogram analysis. Typical chromatograms of drug-free human plasma and urine, standard calibration plasma and urine and treated subject plasma and urine are shown in Figs 2 and 3. The first set of peaks, at retention times of about 5.5 and 6.2 min corresponds to the (S)- and (R)-diastereomeric derivatives of propranolol, respectively. The second set of peaks, at retention times of about 8.8 and 10.1 min corresponds to the (S)- and (R)-diastereomeric derivatives of 4-methylpropranolol (internal standard: I.S.) respectively.

Resolution–sensitivity–linearity. The resolution (R_s) between the enantiomer derivatives of propranolol was 2.0, that between the enantiomer derivatives of I.S. was 2.2, and the peak symmetry was good for all compounds.

The detection limit (signal-to-noise ratio >2) for the assay was about 1 ng ml^{-1} for each propranolol enantiomer.

The standard calibration curves exhibited good linearity for (S) and (R)-propranolol in the range of concentrations tested with correlation coefficients superior to 0.999 for both enantiomers (Table 1).

Accuracy. The interday Relative Standard Deviations (RSD) determined from replicate analysis (n = 6 days) of three plasma and urine standards and of three propranolol forms (non-conjugated, sulphate and glucuronide conjugates) in a human plasma and urine sample after 80 mg racemic propranolol administration are represented in Tables 1 and 2.

The intraday RSD (n = 6) were less than 5% for the same samples (Tables 1 and 2).

The peak area ratios of R/S enantiomer derivatives of these three plasma and urine standards and of the internal standard were greater than 0.99.

Recovery. The recovery of propranolol and I.S. enantiomers from drug-spiked plasma and urine after extraction was determined by comparing peak areas of diastereomeric derivatives from sample extracts with those from directly derivatized solutions of racemic propranolol and I.S. in methanol. The plasmas and urines spiked with 100, 200 and 500 ng ml^{-1} of racemic propranolol and with 1 μg ml^{-1} of I.S.

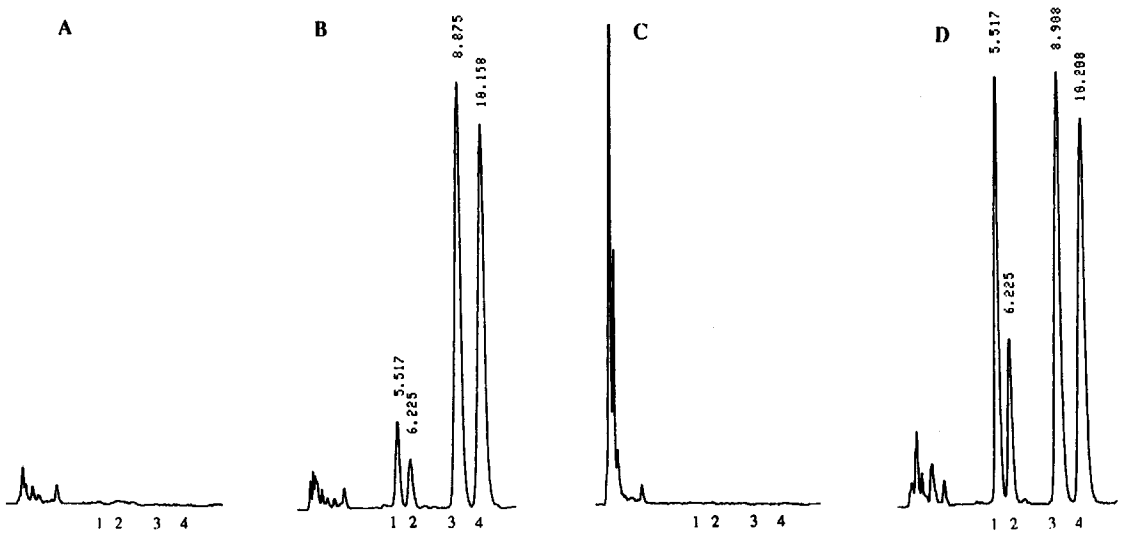


Figure 2
Chromatograms of propranolol enantiomer and internal standard enantiomer derivatives in human plasma and urine. (A) Blank plasma. (B) Unconjugated propranolol enantiomer derivatives in a healthy volunteer plasma. (C) Blank urine after 14 h incubation with glucuronidase–arylsulphatase and saccharic acid 1-4 lactone. (D) Unconjugated and conjugated propranolol enantiomer derivatives in a urine sample diluted ten times and incubated with glucuronidase–arylsulphatase. 1,2: S- and R-propranolol derivatives, respectively. 3,4: S- and R-4-methylpropranolol (I.S.) derivatives, respectively.

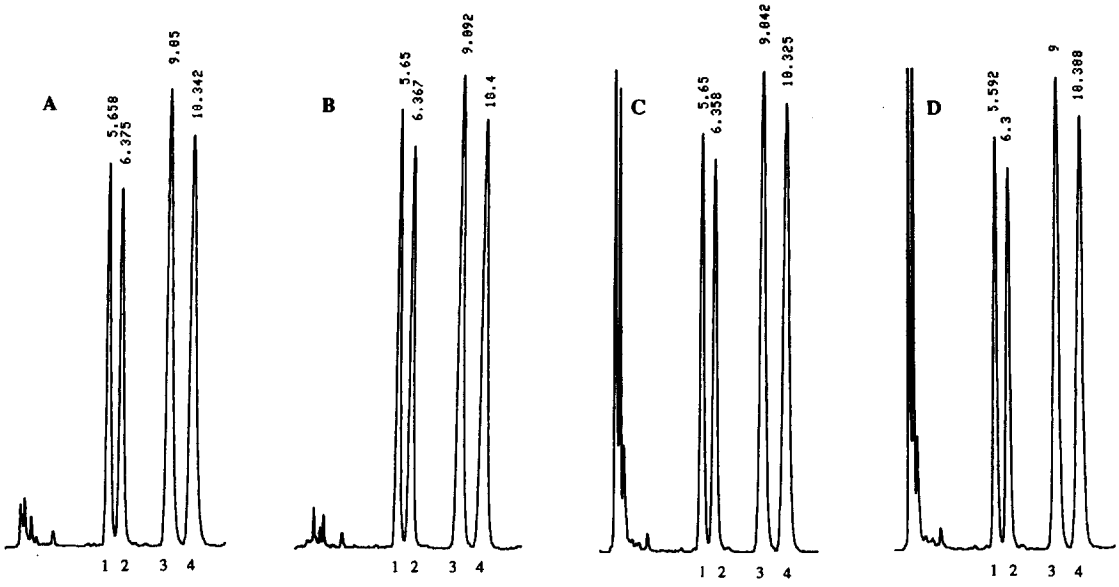


Figure 3
Chromatograms of propranolol enantiomer and internal standard enantiomer derivatives in plasma and urine standards. (A) Plasma spiked with 500 ng ml^{-1} of racemic propranolol after extraction and derivatization. (B) Direct derivatization of a standard containing 500 ng ml^{-1} of racemic drug i.e. 250 ng ml^{-1} of each enantiomer and 1 μg ml^{-1} of racemic I.S. in methanol (no extraction). (C) Urine spiked with 500 ng ml^{-1} of racemic propranolol after extraction and derivatization. (D) Previous urine after 3-day incubation at 4°C. 1,2: S- and R-propranolol derivatives, respectively. 3,4: S- and R-4-methylpropranolol (I.S.) derivatives, respectively.

were recovered from 94 to 98% for propranolol and 95–97% for I.S. Figures 3(A), 3(B) and 3(C) show the identical peak areas from the directly derivatized solution of 500 ng ml^{-1} racemic propranolol and 1 μg ml^{-1} racemic I.S. and from the plasma and urine spiked with the same concentrations of drug and I.S.

Interferences. No endogeneous interfering peaks were observed with drug-free human plasma and urine at the retention times of

Table 1
Intraday and interday accuracy and linearity of propranolol enantiomer standards

		Accuracy of enantiomer standards						Linearity of enantiomer standards*		
		50 ng ml^{-1}		100 ng ml^{-1}		250 ng ml^{-1}				
Samples	Isomer	Intraday† (RSD)	Interday‡ (RSD)	Intraday† (RSD)	Interday‡ (RSD)	Intraday† (RSd)	Interday‡ (RSD)	50 ng ml^{-1}	100 ng ml^{-1}	250 ng ml^{-1}
Plasma	S-propranolol	3.12	3.53	2.50	3.72	2.15	2.80	0.118	0.235	0.589
	R-propranolol	3.25	3.72	2.35	3.10	2.85	3.25	0.119	0.236	0.591
Urine	S-propranolol	2.15	2.10	1.70	2.25	2.10	1.92	0.120	0.238	0.595
	R-propranolol	2.25	2.83	1.85	1.75	1.90	1.80	0.119	0.237	0.594

* Linearity = peak area ratio of S or R-propranolol standard to the corresponding S or R-internal standard (I.S.).
† Intraday: $n = 6$ injections.
‡ Interday: $n = 6$ days.
See text: Results:Accuracy.

Table 2
Intraday and interday accuracy of plasma and urine samples from one healthy volunteer

		Samples from a volunteer after 80 mg racemic drug administration								
		Non-conjugated			Sulphate conjugate			Glucuronide conjugate		
Samples	Isomer	Concentration (ng ml^{-1})	Intraday* (RSD)	Interday† (RSD)	Concentration (ng ml^{-1})	Intraday* (RSD)	Interday† (RSD)	Concentration (ng ml^{-1})	Intraday* (RSD)	Interday† (RSD)
Plasma	S-propranolol	44.0	3.52	4.09	17.8	2.12	2.25	381.2	3.40	3.17
	R-propranolol	38.2	2.83	3.14	7.1	2.53	4.22	107.0	4.06	5.14
Urine	S-propranolol	215.2	2.55	3.0	204.3	3.10	2.60	3316	2.10	2.08
	R-propranolol	182.3	3.10	2.35	85.2	3.20	2.93	1149	2.56	4.12

* Intraday: $n = 6$ injections.
† Interday: $n = 6$ days.
See text: Results:Accuracy.

propranolol and I.S. enantiomer derivatives (Fig. 2A). No metabolite peaks of propranolol enantiomers were found at the retention times of I.S. enantiomer derivatives with plasma or urine samples treated without I.S. The I.S. was pure and showed no interfering peaks at the retention times of propranolol enantiomer derivatives at the concentration used. After incubation of blank plasma or urine with glucuronidase–arylsulphatase and saccharo-lactone, no interfering peaks appeared on the chromatograms (Fig. 2C).

Pharmacokinetic study in humans

The pharmacokinetic data of propranolol enantiomers, their glucuronide and sulphate conjugates in plasma and urine for one of four healthy volunteers are depicted in Figs 4 and 5.

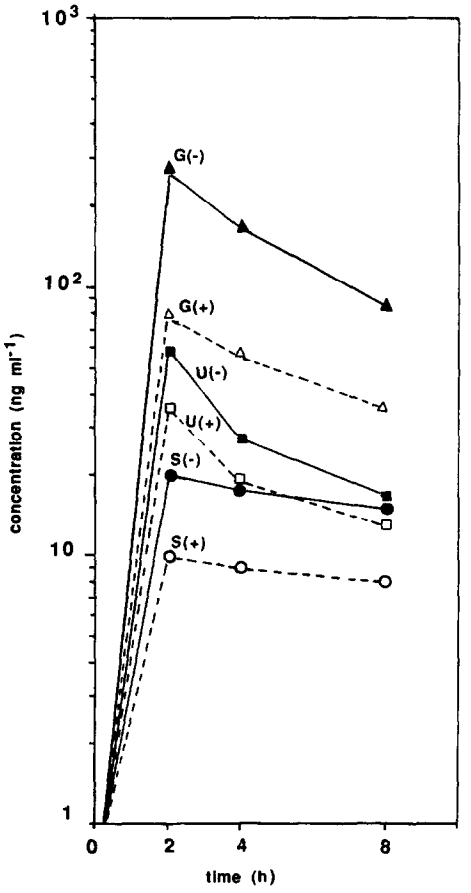


Figure 4
Pharmacokinetic study of unconjugated and conjugated propranolol enantiomers in plasma from one healthy volunteer after oral administration of 80 mg racemic drug. — = S-enantiomer; ---- = R-enantiomer. ■ and U(−) = S(−)-propranolol unconjugate. □ and U(+) = R(+)-propranolol unconjugate. ▲ and G(−) = S(−)-propranolol glucuronide conjugate. △ and G(+) = R(+)-propranolol glucuronide conjugate. ● and S(−) = S(−)-propranolol sulphate conjugate. ○ and S(+) = R(+)-propranolol sulphate conjugate.

In all plasma and urine samples, the concentrations of the S-enantiomer were greater than those of R-enantiomer.

Discussion

Technical study

The technique described in this paper shows improvement over existing HPLC assays using chiral derivatization in several aspects. Most procedures required tedious sample extraction and derivatization [22, 23] or a large sample volume (0.5–1 ml) [9, 19, 20, 21, 23], except the techniques [18, 22] which used 0.1 ml samples. Moreover, chromatographic times for some procedures utilizing either the same derivatizing reagent (PEIC) or the other chiral reagent (tert-butoxycarbonyl-L-leucine anhydride) were long, greater than 30 min [19, 22] or 20 min [9, 18], except the procedures using TAGIT [20] and DATAAN [23] which had chromatographic times less than 10 min. In this procedure, only 100 μl of sample were employed and rapidly extracted with 100 μl of RS-4-methylpropranolol as internal standard and then derivatized with PEIC. The chromatographic time was about 10 min with good separation of propranolol enantiomer derivatives at 5.5 and 6.2 min approximately. The use of a short Novapak C_{18} column (4 μm particles) and of the mobile phase previously described allows even better resolution, sensitivity and chromatographic time than the previously quoted procedures.

The recovery (94–98%) was also better than that of other techniques (78%) [18] and (70%) [23] because of the use of I.S. diluted in methanol, instead of water (other references [9, 19–22] did not indicate their recovery data). Methanol is used to precipitate proteins in the plasma in order to release the propranolol bound to the proteins. The extraction with methanol–diethyl ether (10:90, v/v) in the presence of ammonium hydroxide and the use of a Maximix vortex which can shake four to six tubes at the same time allow a good recovery in a short time. The presence of methanol in diethyl ether is to avoid the emulsion which is one of the causes of the extraction loss.

The diastereomeric derivatives formed by PEIC were very stable. No decomposition or inversion of peaks was observed even after 3 days of storage at 4°C (Fig. 3D). PEIC reagent is very pure; no purification of the

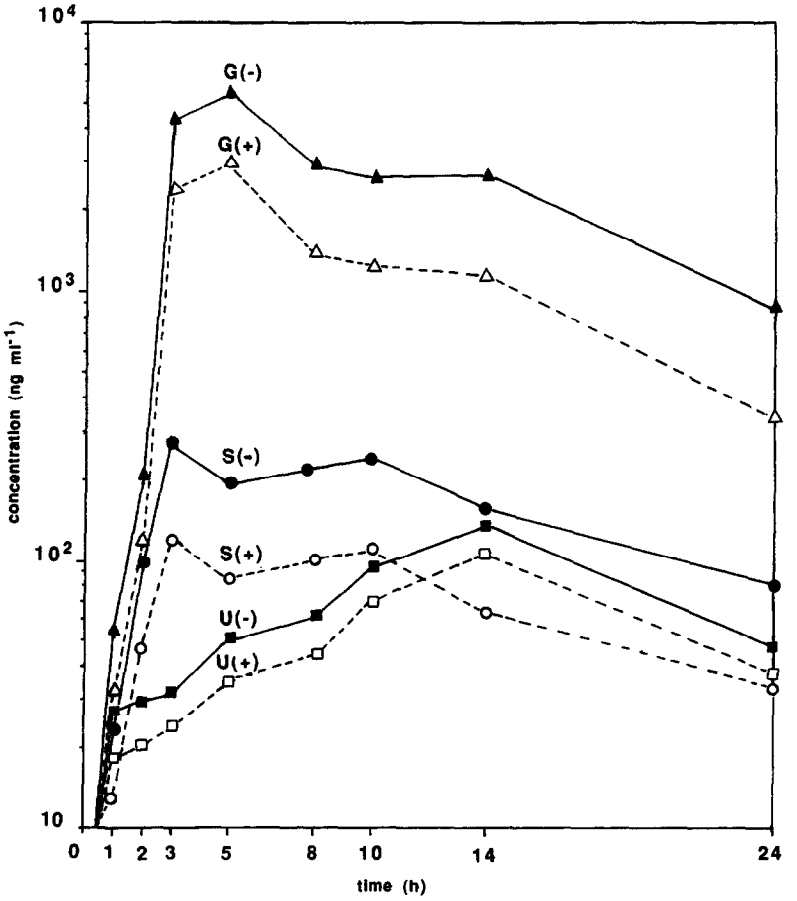


Figure 5
Concentrations of unconjugated and conjugated propranolol enantiomers in urine from one healthy volunteer after oral administration of 80 mg racemic drug as a function of time. — = S-enantiomer; ---- = R-enantimer. ■ and U(−) = S(−)-propranolol unconjugate. □ and U(+) = R(+)-propranolol unconjugate. ▲ and G(−) = S(−)-propranolol glucuronide conjugate. △ and G(+) = R(+)-propranolol glucuronide conjugate. ● and S(−) = S(−)-propranolol sulphate conjugate. ○ and S(+) = R(+)-propranolol sulphate conjugate.

derivatizing reagent before use was necessary at the used concentration. The chromatograms of blank plasma and urine as well as of plasma or urine spiked with a single enantiomer were clean and devoid of interference peaks. However, PEIC must be stored under nitrogen at refrigeration temperature to avoid hydrolysis and oxidation by air. For this reason, it is recommended not to remove the stopper from reagent flask and to withdraw the derivatizing liquid with a syringe. The optimal incubation time of PEIC derivatization was 30 min as recommended by this technique and other procedures [9, 18, 19]. Other reagents used to form propranolol diastereomeric compounds such as tert-butoxycarbonyl-L-leucine anhydride [22], flunoxaprofen chloride (FLOP-Cl) [9] or S(+)benaxoprofen chloride [12] are not commercially available. Moreover, the amide derivatives formed from FLOP-Cl were less stable than the urea derivatives from isocyanate reagents [9].

Some HPLC procedures [19, 20] did not employ an internal standard, thereby reducing the precision and reproducibility of the analysis. An assay was carried out without I.S. by the same procedure; the linearity and reproducibility were less satisfactory with r = 0.975 and RSDs between 10 and 20% because it is difficult to measure exactly the volatile solvent volume. The use of I.S. improved significantly the method accuracy with RSDs less than 5%. In our technique, the choice of the 4-methylpropranolol as internal standard is justified by its physicochemical likeness to that of propranolol, its suitable retention time, its sharp peak symmetry, its good resolution of enantiomer derivatives, its high purity and its

easy availability. Moreover, no eventual metabolite peaks of propranolol derivatives were found at the retention time of I.S.

The incubation time (14 h) for the cleavage of glucuronide and sulphate conjugates in samples was chosen because a time less than 8 h can lead to a loss of 10–15% in comparison with the proposed incubation time.

Pharmacokinetic study in human plasma and urine

As shown in Fig. 4, the maximum plasma concentrations of non-conjugated and conjugated propranolol enantiomers for one of the four healthy volunteers occurred at 2 h, then rapidly decreased at 4 and 8 h after for non-conjugate and glucuronide conjugate and slowly for sulphate conjugate. This pattern was similar for the three other volunteers. The non-conjugated enantiomer levels in plasma were dose-dependent. The sulphate conjugate levels in plasma were generally lower than the non-conjugate levels; in contrast, the glucuronide conjugate concentrations were much greater than the non-conjugate concentrations from three to nine times. The S/R ratios in plasma were generally superior to 1 in all cases, but varied also from individuals. These ratios averaged 1.4 for non-conjugated enantiomers, but were about 2 for sulphate conjugate and about 3 for glucuronide conjugate.

The propranolol sulphate and glucuronide mainly formed in the liver by conjugation of 3-phosphoadenosine-5′-phosphosulphate and uridine-5′-diphospho-glucuronic acid, respectively, with the secondary alcohol of propranolol, are the most important metabolites of propranolol and are the main detoxification forms in man because of their water-solubility. In urine, the concentrations of propranolol sulphate and propranolol glucuronide far exceeded parent drug from 2 to 7 times and from 30 to 150 times, respectively, as shown in Fig. 5 representing propranolol excretion in one of the four healthy volunteers. As in the plasma, the S/R ratio of the three forms in urine varied from 1.2 to 1.5 for non-conjugate, it was about 2 for sulphate conjugate and about 3 for propranolol glucuronide (Fig. 2D).

These pharmacokinetic results in plasma and urine are consistent with those reported by others [3, 26]. The difference between S-enantiomer and R-enantiomer levels in plasma and urine is difficult to explain. According to Walle *et al.* [27] and Olanoff *et al.* [28], the lower plasma binding and greater volume of distribution of R-propranolol contribute to the differences, the main determinant appears to be stereoselective hepatic metabolism of this drug enantiomer. Preferential ring oxidation of R-propranolol [29, 30] and preferential glucuronidation of the S-enantiomer [31] could be the two principal mechanisms, for explaining the higher levels of S-propranolol as compared to its R-antipode in man.

In conclusion, the improved HPLC method described herein is suitable for the therapeutic monitoring and pharmacokinetic study of propranolol enantiomers in human plasma and urine, because of its rapidity, its accuracy, and its sensitivity. Moreover, the sample volume is small. It would be interesting to continue to study propranolol metabolism in order to understand the differences between S-enantiomer and R-enantiomer levels in biological fluids.

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