ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
16 (1998) 991–1003

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Identification and determination of phase I metabolites of propafenone in rat liver perfusate[1]

Weiwei Tan, Qimin Li, Gordon McKay, Hugh A. Semple *

College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, S7N 5C9, Canada

Received 26 October 1996; received in revised form 8 January 1997

Abstract

Propafenone (PF) is a class 1C antiarrhythmic agent. To study the mechanisms of PF interactions with dietary nutrients in isolated, perfused rat livers, metabolites of PF in liver perfusate were identified and an analytical method was developed for these metabolites plus parent drug. Identification of phase I metabolites was performed using HPLC/MS equipped with a Lichrospher RP-18 column and tandem mass spectrometry (MS/MS) with electrospray and atmospheric pressure chemical ionizations. Three major metabolite peaks, whose protonated molecular ions were m/z 358, 358 and 300, were identified as a propafenone derivative hydroxylated in the ω-phenyl ring (ω-OH-PF), 5-hydroxypropafenone (5-OH-PF), and *N*-despropylpropafenone (*N*-des-PF). The levels of ω-OH-PF, 5-OH-PF, *N*-des-PF and PF were determined simultaneously by HPLC with UV detection at 210 nm and a mobile phase of 0.03% triethylamine and 0.05% phosphoric acid in water-acetonitrile-methanol (45:20:35, v/v/v) after extraction with 5 ml diethyl ether at pH 10.0 and evaporation of solvent under nitrogen. The results revealed that ω-OH-PF, which was not found in humans, was the major metabolite of PF in rat liver perfusate, not 5-OH-PF which is the major metabolite in human plasma.

Keywords: Propafenone; Drug metabolism; HPLC; Mass spectrometry; Perfused rat liver

1. Introduction

Propafenone (PF), 2′ - (2 - hydroxy - 3 - propylamino-propoxy)-3-phenylpropiophenone, is a potent antiarrhythmic drug being widely used in the treatment of ventricular and supraventricular arrhythmias [1]. Like some other high hepatic first-pass drugs, e.g., metoprolol and propranolol, propafenone shows dramatically increased bioavailability when given with food, even though it is completely absorbed after an oral dose [2,3]. In order to carry out studies on this 'food effect', which may be related to interaction between nutrients and drugs in the liver, metabolism of propafenone has been studied in the isolated, perfused rat liver.

Propafenone undergoes extensive hepatic oxidative metabolism in the dog and man. Less than

* Corresponding author: Tel.: + 1 306 9666365; fax: + 1 306 9666377; e-mail: hugh.semple@usask.ca

[1] Presented at the Analysis and Pharmaceutical Quality Section of the Eleventh Annual American Association of Pharmaceutical Scientists Meeting, October 1996, Seattle, Washington, USA.

0731-7085/98/$19.00
PII S0731-7085(97)00055-1

1% of the dose was recovered as unchanged substance in urine and bile [4,5]. After oral doses of deuterated propafenone in man, two hydroxylated propafenone derivatives, 5-hydroxypropafenone (5-OH-PF) and 5-hydroxy-4-methoxy-propafenone (5-OH-4-OCH_3-PF) were described as the two major phase I metabolites in the samples of urine, bile, feces and plasma [4,6]. Moreover, *N*-despropylpropafenone (*N*-des-PF), a *N*-dealkylated metabolite, was found to accumulate in the plasma of patients during chronic therapy with the parent drug [7]. All of these three major metabolites have activity comparable to that of propafenone [4,8,9]. In dog urine and bile samples, the major phase I metabolites, 5-hy-droxypropafenone and 4′-hydroxypropafenone (4′-OH-PF), accounted for about 15% of the dose for each [4,6], whereas 5-hydroxy-4-methoxypropafenone and *N*-despropylpropafenone are only of minor importance in quantity. The structures of propafenone and its major phase I metabolites in dog and man are shown in Fig. 1.

Although major phase I metabolites of propafenone have been identified in humans and dogs, no metabolism studies have been reported in rats or other animals. The present paper describes the identification of phase I metabolites of propafenone in the effluent samples from the isolated, perfused rat liver by high performance liquid chromatography/mass spectrometry (HPLC/MS) and tandem mass spectrometry (MS/MS) methods. Finally, the levels of these metabolites were determined simultaneously by a simple and convenient HPLC method.

2. Experimental

2.1. Chemicals

All solvents used for extraction and preparation of HPLC mobile phase were HPLC grade obtained form BDH Chemicals Canada (Edmonton, AB, Canada). Other chemicals used were commercial analytical grade purchased from BDH. Propafenone hydrochloride (HCl) and Type H-1 β-glucuronidase were obtained from Sigma Chemical (St. Louis, MO, USA). Authentic standards of propafenone-HCl (Lu 29007), 5-hydroxypropafenone-HCl (Lu 40545), *N*-despropylpropafenone-fumarate (Lu 48686) and internal standard (Li 1115-HCl, the *N*-ethyl analogue of PF) were kindly supplied by Knoll AG, Ludwigshafen, Germany. The structures of these compounds are shown in Fig. 1.

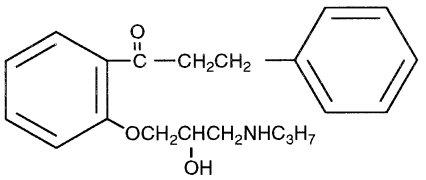
2.2. Liver perfusion

The surgical procedure and the perfusion apparatus were identical to those previously described [10]. Following an overnight fast, male Sprague-Dawley rats weighing 220–250 g were anesthetized by halothane inhalation, the vena cava and hepatic portal vein were catheterized, and the livers were isolated and placed in a perfusion cabinet. The rat livers were perfused via the hepatic portal vein with oxygenated perfusate (5% CO_2 and 95% O_2) at a flow rate of 25–30 ml min^{-1} liver. The perfusate contained 2 g l^{-1} of glucose and 20 or 50 μg ml^{-1} of propafenone in Krebs bicarbonate solution, buffered to pH 7.4. A portion of the effluent from the vena cava was passed through the microelectrode flow-through cell of an oxygen monitor (YSI model 5300, Fisher Scientific, Edmonton, AB, Canada), while the major portion was collected for analysis. The entire circuit was temperature controlled at 37°C. Perfusate samples were collected during the time interval of 60–100 min after perfusion with drug, in which propafenone was known to be at steady state (unpublished data). Aliquots of perfusate samples were stored at −20°C until analysis.

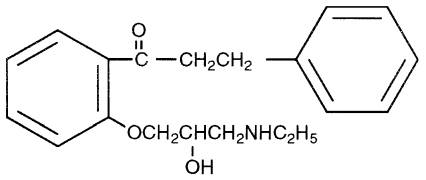
2.3. Sample preparation

The phase I metabolites were extracted with diethyl ether. Perfusate samples (1 ml) were vortex mixed with 5 ml of diethyl ether for 10 min. After centrifugation at 1200 × *g* for 10 min, the organic layer was transferred to a clean borosilicate glass tube and evaporated to dryness under a stream of N_2 at room temperature. The residues were reconstituted in 200 μl of HPLC mobile phase, and injected into the HPLC system.

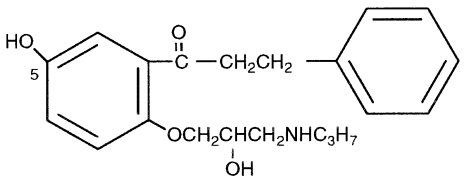
Each peak obtained from HPLC with UV detection was purified by a Spe-ed cartridge packed



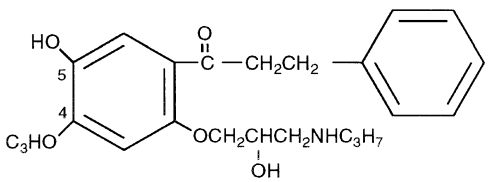
propafenone



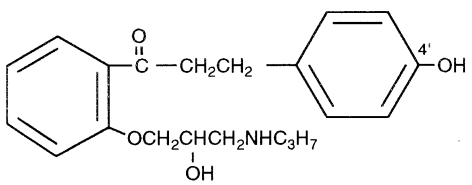
internal standard



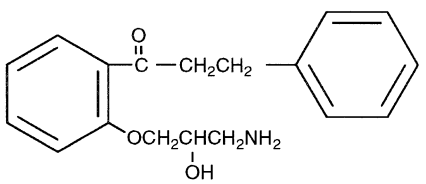
5-hydroxy-propafenone



5-hydroxy-4-methoxy-propafenone



4'-hydroxy-propafenone



N-despropyl-propafenone

Fig. 1. Structural formulae of propafenone and its metabolites and internal standard.

with solid phase C_{18} (Applied Separations, Bethlehem, PA, USA). The cartridge was conditioned by eluting with 2 ml of acetonitrile, 2 ml of methanol, and 2 ml of distilled water. Each fraction collected from the HPLC was blown under a flow of nitrogen to evaporate organic solvents, and then lyophilized. The residue was reconstituted in 1 ml of water, and applied on the conditioned cartridge. After the samples had adsorbed to the solid phase, the cartridge was washed with 3 ml of water to remove salts contained in the samples. Finally the metabolite fraction was eluted with 1 ml of methanol, which was evaporated under a stream of nitrogen. The residue was reconstituted in 1 ml of methanol for injection into the MS/MS spectrometer.

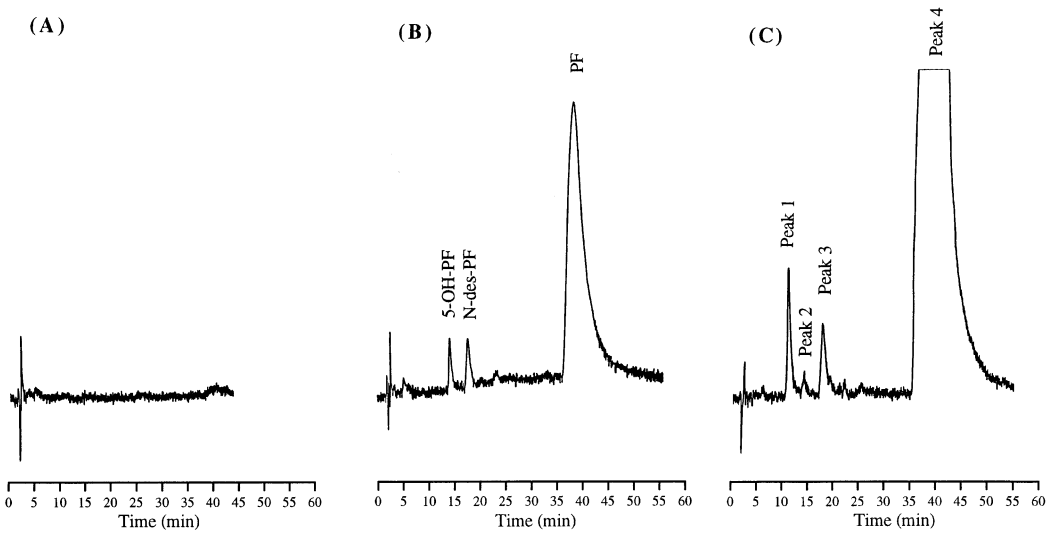


Fig. 2. HPLC/UV chromatograms of extracts from (A) blank rat liver perfusate; (B) blank rat liver perfusate spiked with reference standards; (C) rat liver perfusate after infusion of 50 μg ml^{-1} PF.

2.4. Chromatographic methods

2.4.1. HPLC conditions

Propafenone and its phase I metabolites were separated by an HPLC system with a mobile phase of ammonium acetate (0.005 M)-acetonitrile-methanol (50:15:35, v/v/v), apparent pH (pH*) adjusted 2.90 with trifluoroacetic acid, pumped at a flow rate of 1 ml min^{-1}. The HPLC system consisted of a Waters 510 pump, a Waters 490 programmable multiwavelength detector set at 210 nm (Millipore-Waters, Mississauga, ON, Canada), a Model 7125 syringe loading sample injector with 200 μl loop (Rheodyne, Cotati, CA, USA), and a 250 × 4 mm I.D., 5 μm particle size Lichrospher RP-18 column (E. Merck, Darmstadt, Germany). The data were recorded using a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan).

2.4.2. HPLC/MS and MS/MS

HPLC/MS and MS/MS spectra were obtained using a VG Quattro-II triple quadrupole mass spectrometer equipped with an electrospray ion source (Micromass, Altrincham, UK). HPLC/MS was performed with an atmospheric pressure chemical ionization (APCI) interface in positive mode. The probe temperature and corona discharge pin were maintained at 500°C and 3.5 kV, respectively. Product ion spectra were obtained by positive electrospray (+ES) MS/MS with collision-induced dissociation (CID). The collision energy, E_{lab}, was 20 eV, and the argon pressure was set at 1.0×10^{-3} mBar. The HPLC solvent delivery system utilized a model 140A dual syringe pump (Applied Biosystems, Mississauga, ON, Canada) fitted with a Rheodyne 7125 valve loop injector equipped with a 20 μl sample loop. The separation column and HPLC conditions used in HPLC/MS system are described as above.

2.5. Quantitation of propafenone and its metabolites in rat liver perfusate

To 1 ml of rat liver perfusate were added 30 μl of internal standard solution (100 μg ml^{-1}). The drug, metabolites and internal standard were extracted with 5 ml of diethyl ether and 2 ml of sodium bicarbonate buffer (0.05M, pH 10.0). The extracted samples were reconstituted in 200 μl of mobile phase, and 20 μl aliquots were injected into the HPLC system with a mobile phase of water-acetonitrile-methanol (45:20:35, v/v/v), containing 0.03% (v/v) triethylamine and 0.05% (v/v)

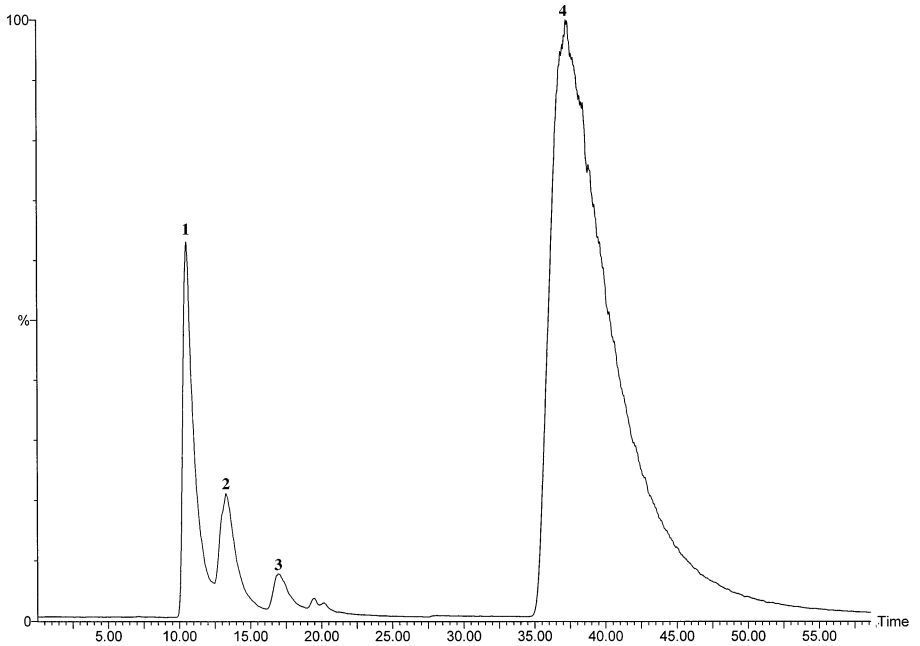


Fig. 3. HPLC/MS total ion chromatogram (m/z 200–450) of extract from rat liver perfusate after infusion of 20 μg ml^{-1} PF.

of concentrated phosphoric acid, pH* 3.10. Standard samples were prepared in 1 ml of blank rat liver perfusate at concentrations of 50, 100, 200, 400, 800 and 1000 ng ml^{-1} of 5-hydroxypropafenone and *N*-despropylpropafenone, and 5, 6, 8, 10, 12 and 15 μg ml^{-1} of propafenone. Calibration curves were obtained by plotting peak height ratios of drug/internal standard vs. drug concentrations in standard samples.

Then, 1 ml samples of liver perfusate collected during 100–105 min after infusion with 20 μg ml^{-1} propafenone were incubated with 5000 units of β-glucuronidase (contains 338 000 U g^{-1} of β-glucuronidase and 16 000 U g^{-1} of sulfatase) at 37°C for 4 h, and the levels of propafenone and its phase I metabolites were determined. Quality control (QC) samples duplicated at two different concentrations (close to the highest and lowest standard curve concentrations) of PF and its metabolites in the range of the calibration curves were prepared with the analyst blinded, and incorporated into each batch.

3. Results

3.1. Identification of metabolites

A typical HPLC/UV chromatogram of extract from rat liver perfusate after infusion of PF (Fig. 2C) gave four major peaks which were not present in the chromatogram of blank liver perfusate extract (Fig. 2A). Of the four peaks, assigned as peaks 1, 2, 3 and 4, peaks 2, 3 and 4 had the same retention time values as those obtained from authentic 5-OH-PF, *N*-des-PF and PF (Fig. 2B). The liver perfusate extract was then analyzed by HPLC/MS. The HPLC/MS total ion chromatogram scanned from m/z 200–450 (Fig. 3) demonstrated similar chromatographic peaks labeled 1, 2, 3, and 4 as those previously obtained in the HPLC/UV analysis. The positive ion background-subtracted mass spectra of these peaks are reported in Fig. 4. During APCI ionization very little fragmentation was observed, and only probable protonated molecular ions (MH^+) were evidenced, respectively, at m/z 358, 358, 300 and 342,

together with apparently natriurated molecular ions (MNa^+). On the basis of their molecular weights and HPLC retention time values, peaks 2, 3 and 4 were tentatively identified as 5-OH-PF, *N*-des-PF and PF, respectively. Peak 1, however, did not correspond to any authentic compounds, although it is suggestive of a hydroxylated analogue of PF because of the inferred molecular weight of 357 Da, 16 Da above that of parent drug and the same nominal m/z as that of 5-OH-PF.

In order to obtain structural information about peak 1, the appropriate fractions corresponding to each peak from the HPLC chromatograms were collected, desalted, and then analyzed by direct loop injection into mass spectrometer operated in the MS/MS mode. Pre-selection of the precursor ions, m/z 358, 300 and 342, was carried out in each peak and the corresponding CID product ion spectra was recorded. The CID product ion spectra of peaks 2, 3, and 4, which are shown in Fig. 5A, Fig. 6 and Fig. 7, respectively, gave further confirmation of the identities to 5-OH-PF, *N*-des-PF and PF, respectively, since they were practically superimposable with the spectra of the authentic compounds. The product ion spectrum of peak 1 (Fig. 8A), however, showed distinctive differences from that of peak 2, indicating that these two hydroxylated PF metabolites with pseudomolecular ions at m/z 358 are isomers. The product ion observed at m/z 107, could be justified if hydroxylation occurred at the terminal phenyl ring. Similarly the product ions at m/z 234 and 175, could be justified from the unhydroxylated disubstituted phenyl ring (Fig. 8B), strongly suggesting that the site of hydroxylation in the material eluting under peak 1 must be on the ω-phenyl ring. This derivative hydroxylated in the ω-phenyl ring (ω-OH-PF) should correspond to 4′-OH-PF, according to Hege et al. [6]. In contrast to the product ion spectrum of peak 1, 5-OH-PF showed diagnostic ions at m/z 91 generated from the unhydroxylated terminal phenyl ring and m/z 281 generated from the hydroxylated middle phenyl ring (Fig. 5A), and no product ions at m/z 234, 175 and 107.

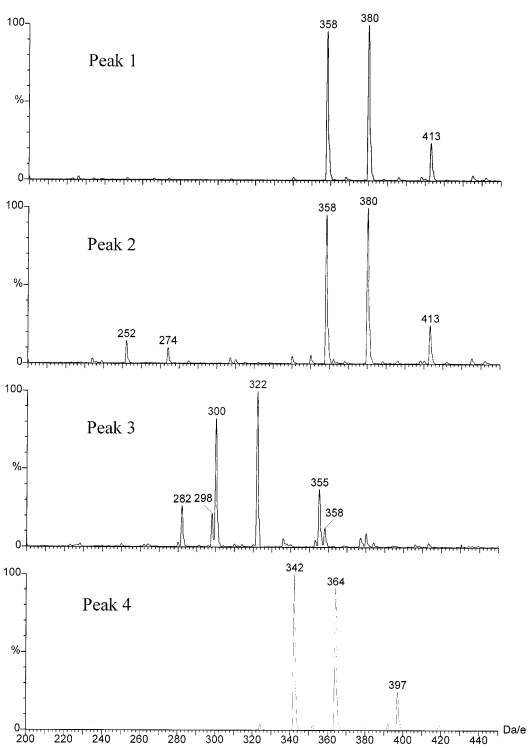


Fig. 4. Positive ion background-subtracted mass spectra of peaks 1, 2, 3 and 4.

3.2. Quantitation of propafenone and its metabolites

The HPLC chromatograms under the conditions used to quantitate PF and its metabolites are shown in Fig. 9. Because no standard reference compound for peak 1 was available, the fraction collected from HPLC and identified by MS/MS was used as a standard reference to confirm the retention time of ω-OH-PF after the mobile phase was modified. The quantity of the metabolite from the collected fraction was insufficient to prepare a standard solution for a calibration curve, therefore the peak height ratios of drug/internal standard were used to reflect the levels of this metabolite in rat liver perfusate.

The extraction efficiency of diethyl ether at pH 10.0 was evaluated by comparing the peak height ratios of the extracted spiked samples to the unextracted samples which were directly injected into the HPLC. The mean recoveries ($n = 5$) after extraction were 96.6% for 5 μg ml^{-1} of PF, 95.4%

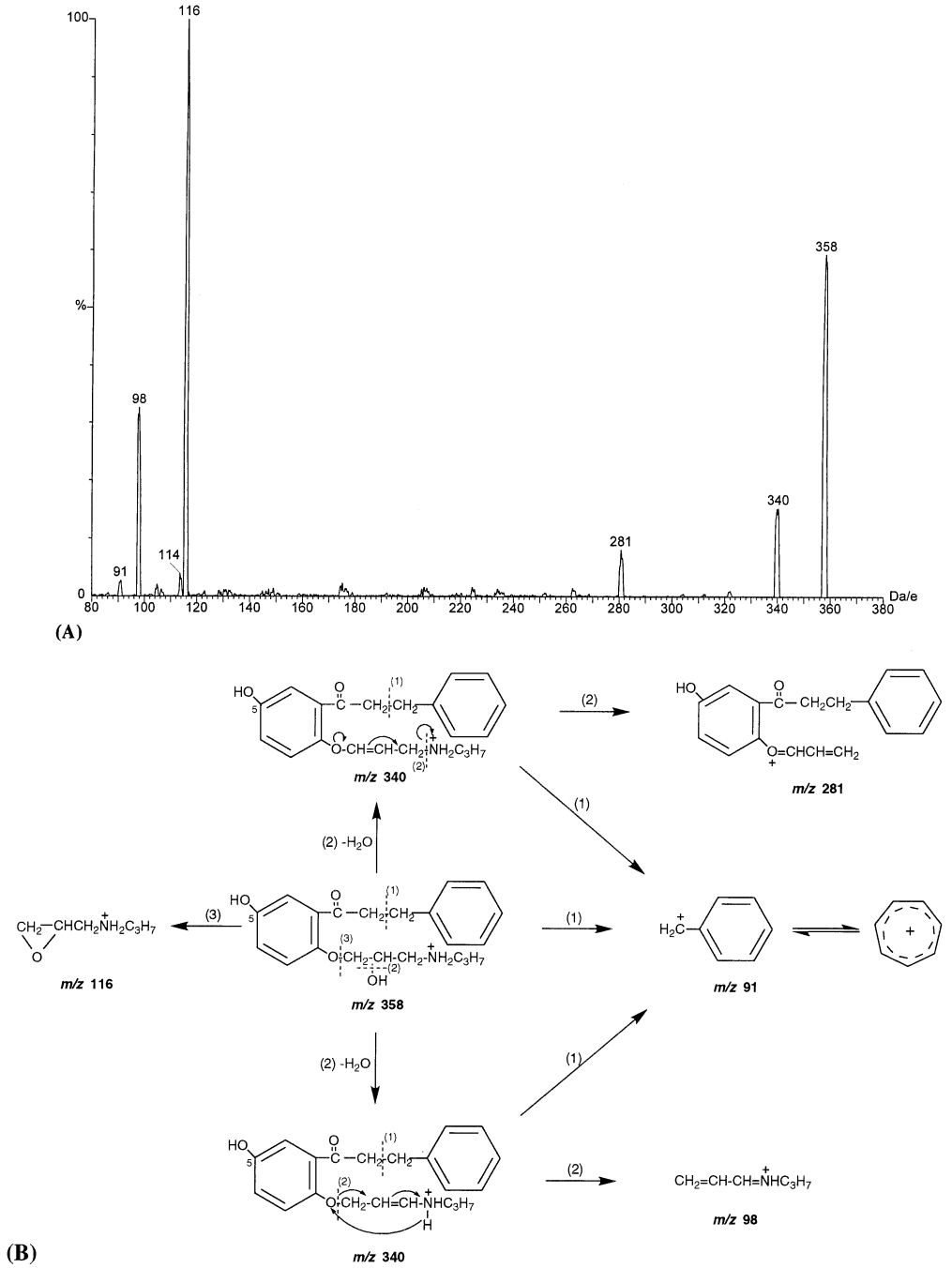


Fig. 5. Product ion analysis for peak 2. (A) product ion mass spectrum of the protonated molecule of peak 2 at m/z 358; (B) proposed fragmentation pattern for peak 2.

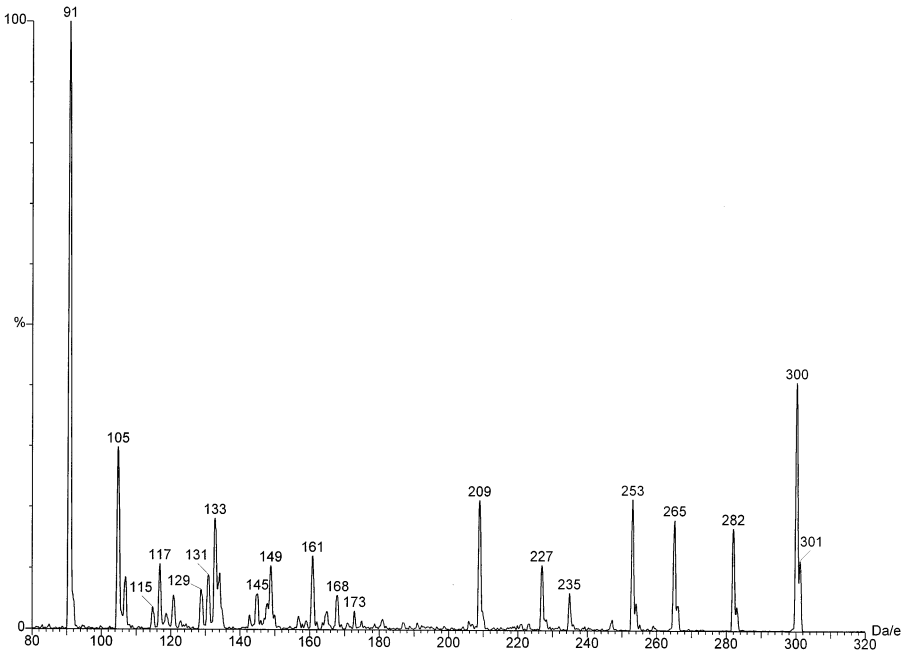


Fig. 6. Product ion mass spectrum of the protonated molecule of peak 3 at m/z 300.

for 500 ng ml^{-1} of 5-OH-PF and 93.6% for 500 ng ml^{-1} of *N*-des-PF, respectively. The calibration curves for these three analytes are summarized in Table 1. All the concentrations of QC samples were within 12% error of the nominal concentrations.

The total concentrations of PF, 5-OH-PF and *N*-des-PF in rat liver perfusate after infusion of 20 μg ml^{-1} (53.0 μM) of PF were determined after cleavage of conjugates, which were 12 657 ng ml^{-1} (33.5 μM) ± 4.54%, 948.6 ng ml^{-1} (2.41 μM) ± 3.80% and 632.3 ng ml^{-1} (1.52 μM) ± 1.34% (mean ± R.S.D., $n = 3$), respectively. Peak height ratios of peak 1 and 5-OH-PF are 2.145 ± 2.2% and 0.429 ± 3.6%, respectively.

4. Discussion

Studies on propafenone metabolism in humans [5–7] have revealed that 5-OH-PF, *N*-des-PF and 5-OH-4-OCH_3-PF are the three major phase I metabolites in plasma. In dogs [4,6], 5-OH-PF and 4′-OH-PF were found to be the two major phase I metabolites, while other metabolites, such as 5-OH-4-OCH_3-PF, 4′-hydroxy-3′-methoxy-propafenone (4′-OH-3′-OCH_3-PF) and *N*-des-PF were present in lower quantities. We have demonstrated that at least three phase I metabolites, ω-OH-PF, 5-OH-PF and *N*-des-PF were produced in isolated, perfused rat livers, and ω-OH-PF was the main metabolite. In all these three species, aromatic ring hydroxylation commonly dominates the phase I metabolism, whereas *N*-dealkylation reactions are of secondary importance. Most drugs containing aromatic moieties are susceptible to aromatic oxidation, so the ring hydroxylation of propafenone is not surprising. These species, however, showed different regioselectivity of hydroxylation in the aromatic rings. Compared to humans, in which hydroxylation is only favored in the disubstituted phenyl ring, the rat and dog have broader spectra of hydroxylated metabolites as they are capable of hydroxylating both phenyl rings, and the rat metabolic enzymes appear more selective for the ω-phenyl ring. The metabolism of diprafenone (Fig. 10), a new antiarrhythmic agent with a chemical structure analogous to PF, has also been studied in Sprague-Dawley rats after intravenous and oral administration [11]. A main metabolite

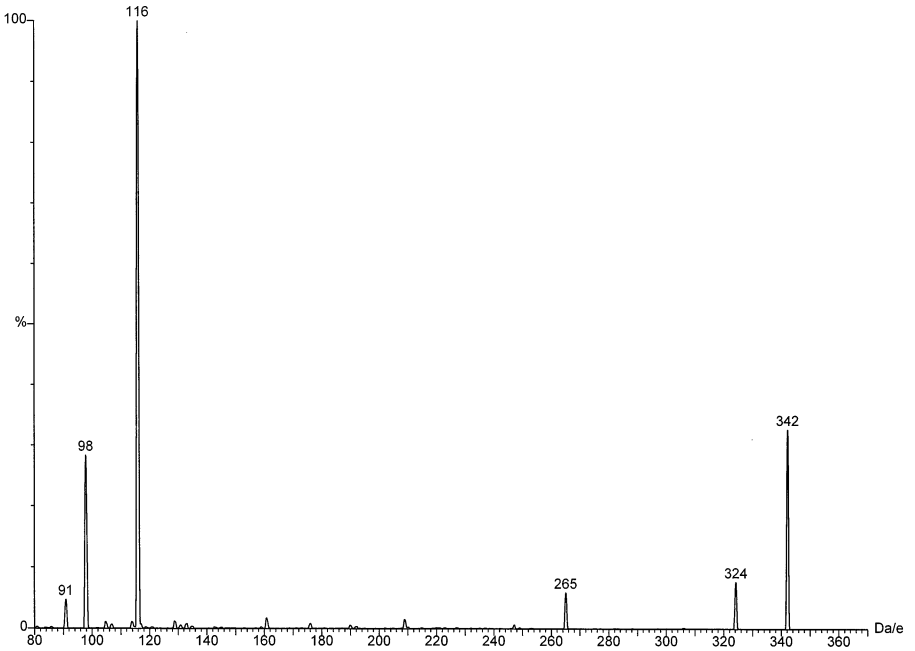


Fig. 7. Product ion mass spectrum of the protonated molecule of peak 4 at m/z 342.

other than 5-hydroxydiprafenone was found but not identified. This unknown abundant metabolite of diprafenone may be a derivative hydroxylated in the terminal phenyl ring based on our studies on metabolism of PF, which should be parallel to that of diprafenone.

Different regioselectivity of hydroxylation between species has also been demonstrated in some other drugs containing aromatic ring(s), e.g., propranolol (Fig. 11). One major metabolic pathway of propranolol is naphthalene ring hydroxylation, which gives rise to several regioisomeric hydroxylated metabolites in humans and rats [12]. Of these hydroxylated metabolites, 4-hydroxypropranolol and 5-hydroxypropranolol are two major ones in humans, while 4-hydroxypropranolol and 7-hydroxypropranolol are more predominant in rats [12]. Based on the well-established cytochrome *P*-450 system involved in propranolol metabolism, the positional difference in naphthalene ring hydroxylation between rats and humans may be attributed to the different regioselectivity of different cytochrome *P*-450 isoenzymes involved in these two species. CYP2D6 is responsible for 4, 5, and 7-hydroxylation in humans [13] and the CYP2D subfamily is responsible in rats [14]. Similarly to the situation with propranolol, it was demonstrated that 5-hydroxylation of PF in humans was mediated via CYP2D6 [15], a debrisoquine 4-hydroxylase which does not exist in rats. Therefore, the differences in the CYP2D subfamily between rats (or dogs) and humans may explain the positional difference in the phenyl ring hydroxylation of PF.

Metabolites for identification were extracted into diethyl ether directly from liver perfusate of pH 8–9. This implies that only basic and neutral metabolites, but probably not acidic metabolites were extracted. Since acidic metabolites of PF are secondary metabolites which were present in very small quantities in dog [4] and human [5], however, it is postulated that acidic metabolites would be formed in much lower quantities in the single-pass isolated rat liver. In addition to the monohydroxylated compounds, dihydroxylated and other metabolites could also be produced in rat livers, but in small quantities.

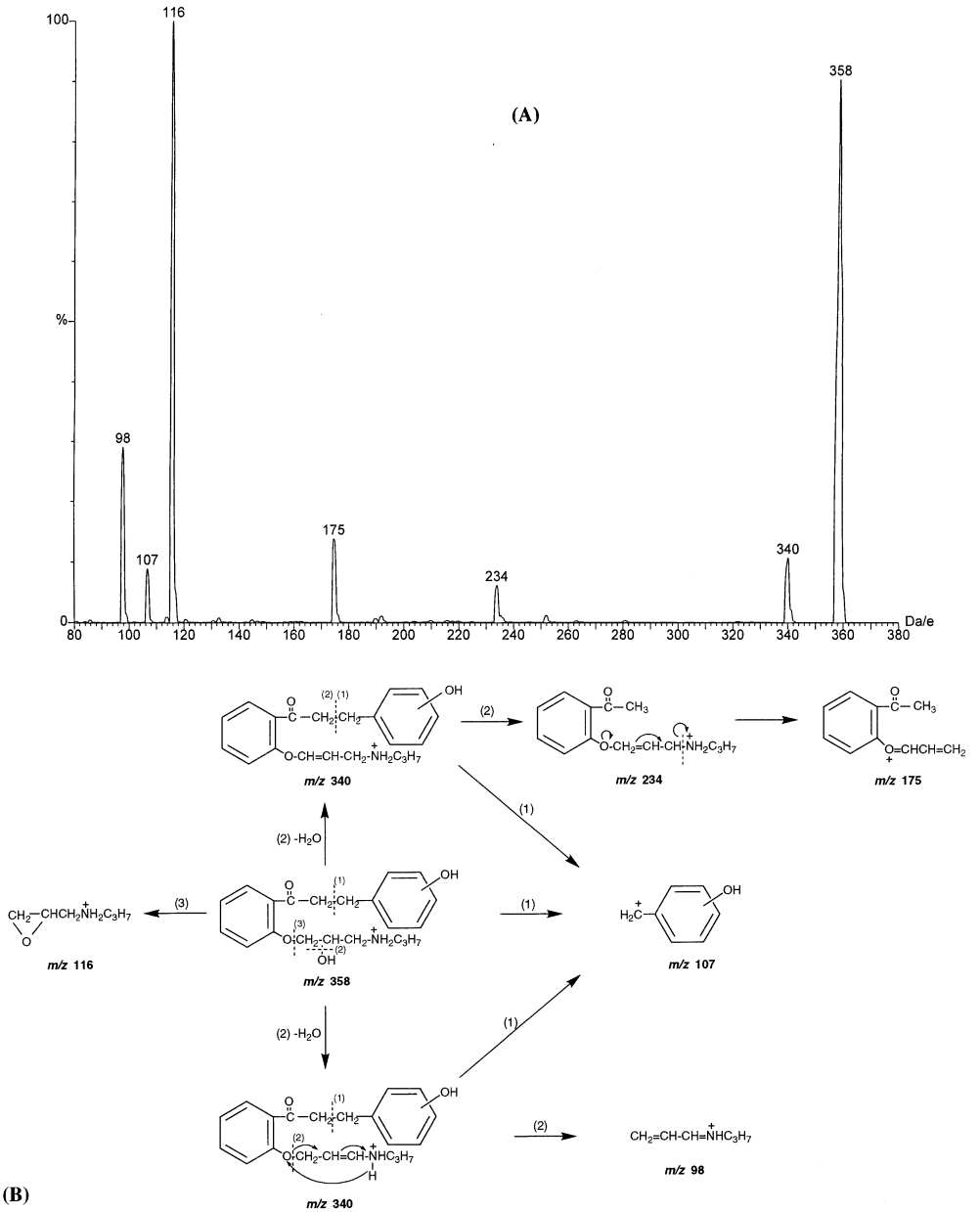


Fig. 8. Product ion analysis for peak 1. (A) product ion mass spectrum of the protonated molecule of peak 1 at m/z 358; (B) proposed fragmentation pattern for peak 1.

Although we could not find direct evidence to confirm the position(s) of hydroxylation in the ω-phenyl ring because no standard references were available, 4′-OH-PF is proposed as the most plausible chemical structure for the metabolite hydroxylated on the ω-phenyl ring. This proposition is based on (1) the report by Hege [6] in which the possibility of hydroxylation on the 2′ or

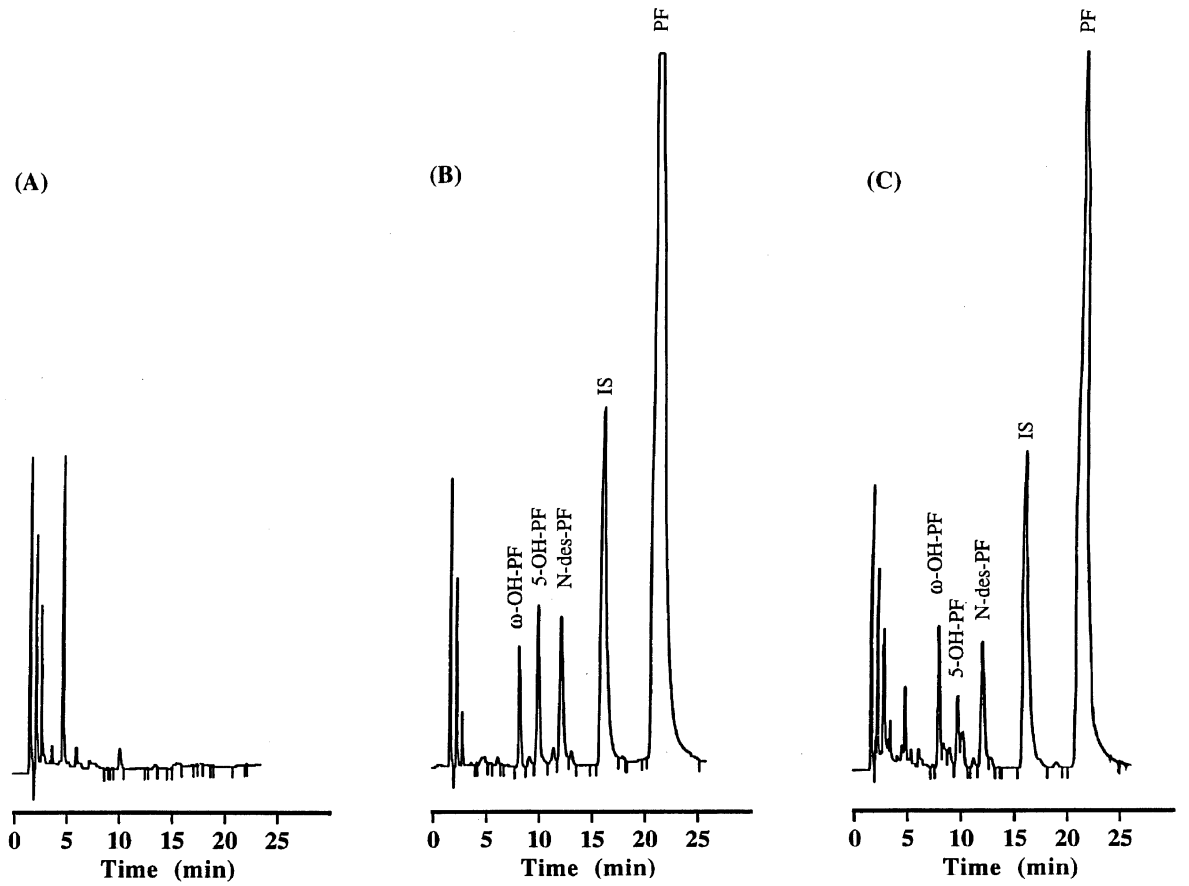


Fig. 9. HPLC chromatograms for determination of PF and its metabolites. (A) blank rat liver perfusate; (B) blank rat liver perfusate spiked with reference standards, internal standard (IS) and the collected fraction of peak 1; (C) rat liver perfusate after infusion of 20 μg ml^{-1} PF.

3′-positions of the ω-phenyl ring was ruled out; and (2) a preference for the para position in metabolic arenoxidation [16]. Nevertheless, hydroxylation at other positions could not be excluded for certain.

Based on the identification of propafenone and its metabolites, the levels of these compounds in liver perfusate can be determined simultaneously by a rapid and convenient conventional HPLC method. The HPLC method is sensitive and accurate for determining the concentrations of PF and its metabolites, although the concentration range chosen was quite high, due to the high concentrations of PF used in our experiments. Unfortu-

Table 1
Calibration curve parameters for quantitation of PF, 5-OH-PF and *N*-des-PF in rat liver perfusate using HPLC with UV detection

Analyte	Retention time (min)	Range (ng ml^{-1})	Slopes ($\times 10^{-4}$)	Intercepts	*r*	Mean accuracy(%) ± R.S..D
PF	21.3	5000–15 000	1.551	0.0646	0.999	101.1 ± 6.23%
5-OH-PF	10	50–1000	4.353	0.1607	0.999	103.2 ± 1.62%
N-des-PF	12.2	50–1000	3.807	0.0333	0.998	102.0 ± 3.83%

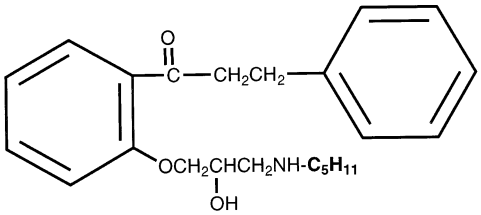


Fig. 10. Structural formula of diprafenone.

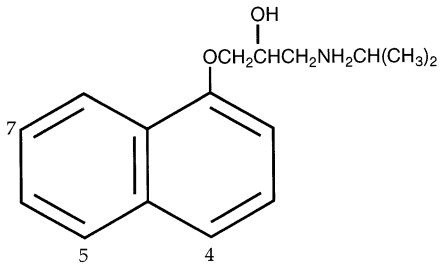


Fig. 11. Structural formula of propranolol.

nately, no standard reference for ω-OH-PF was available, so we used the peak height ratio to record changes in its concentrations in rat liver perfusate. Extraction with diethyl ether at pH 10.0 provided high recoveries of PF, 5-OH-PF and *N*-des-PF from liver perfusate. The mobile phase was modified by addition of triethylamine and phosphoric acid, which are not suitable for mass spectrometry for identification, instead of ammonium acetate and trifluoroacetic acid. The modified mobile phase provided better peak shapes, shorter retention time values, and therefore higher sensitivity for all peaks (Fig. 9).

Quantitation of PF and its metabolites after conjugate cleavage showed that total phase I metabolites accounted for about 36.8% of the 20 μg ml^{-1} of PF perfused through rat liver. Compared to dogs and humans, the smaller proportion of phase I metabolism may have been the result of saturation of hepatic uptake or metabolism at the extremely high inlet concentrations employed. Assuming that ω-OH-PF would give a similar absorbance value to that of 5-OH-PF at 210 nm, the calibration curve for 5-OH-PF was adapted to calculated the approximate concentrations of ω-OH-PF in rat liver perfusate. The results showed that ω-OH-PF, 5-OH-PF and *N*-des-PF accounted for about 63.6, 12.4 and 7.7% of total phase I metabolites, respectively, showing that ω-OH-PF is the major phase I metabolite in rat liver perfusate, whereas other phase I metabolites only accounted for a small percentage. It was observed that the ratio of these three metabolites is changed with sample collection time and with PF inlet concentration in perfused rat livers, but ω-OH-PF was consistently eluted at higher levels than those of the other two metabolites.

5. Conclusions

The metabolism of propafenone in rats resembles that in dogs, but not in humans. *N*-dealkylation and hydroxylation in the middle and terminal phenyl rings are the major metabolic pathways in isolated, perfused rat livers. The ω-phenyl ring hydroxylated metabolite makes up the largest proportion. The difference in phase I metabolism of PF in the rat may limit the usefulness of this species as a model of human PF metabolism. The position of hydroxylation in the ω-phenyl ring needs to be further elucidated because no standard references were available.

Acknowledgements

This work was supported by the Medical Research Council of Canada (DG-405) and the Heart and Stroke Foundation of Saskatchewan.

References

[1] D.W.G. Harron, R.N. Brogden, Drugs 34 (1987) 617–647.
[2] A. Melander, D. Lalka, A.J. McLean, Pharmacol. Ther. 38 (1988) 253–267.
[3] J.E. Axelson, G.L. Chan, E.B. Kirsten, W.D. Mason, R.C. Lanman, C.R. Kerr, Br. J. Clin. Pharmacol. 23 (1987) 735–741.
[4] H.G. Hege, H. Lietz, J. Weymann, Arzneim.-Forsch./Drug Res. 39 (1984) 972–979.
[5] H.G. Hege, M. Hollmann, S. Kaumeier, H. Lietz, Eur. J. Drug Metab. Pharmacokinet. 9 (1984) 41–55.
[6] H.G. Hege, H. Lietz, J. Weymann, Arzneim.-Forsch./Drug Res. 36 (1986) 467–474.

[7] R.E. Kates, Y.G. Yee, R.A. Winkle, Clin. Pharmacol. Ther. 37 (1985) 610–614.
[8] K. Oti-Amoako, S. Vozeh, H.-R. Ha, F. Follath, J. Cardiovasc. Pharmacol. 15 (1990) 75–81.
[9] K.A. Thompson, D.H.S. Iansmith, L.A. Siddoway, R.L. Woosley, D.M. Roden, J. Pharmacol. Exp. Ther. 244 (1988) 950–955.
[10] H.A. Semple, F. Xia, Drug Metab. Dispos. 22 (1994) 822–826.
[11] F. Brunner, U. Kolmayr, W.R. Kukovetz, Arzneim.-Forsch./Drug Res. 39 (1989) 1430–1435.
[12] T. Walle, J.E. Oatis Jr., U.K. Walle, D.R. Knapp, Drug Metab. Dispos. 10 (1982) 122–127.
[13] Y. Masubuchi, S. Hosokawa, T. Horie, T. Suzuki, S. Ohmori, M. Kitada, S. Narimatsu, Drug Metab. Dispos. 22 (1994) 909–915.
[14] Y. Masubuchi, N. Kagimoto, S. Narimatsu, S. Fujta, T. Suzuki, Drug Metab. Dispos. 21 (1993) 1012–1016.
[15] H.K. Kroemer, G. Mikus, T. Kronbach, U.A. Meyer, M. Eichelbaum, Clin. Pharmacol. Ther. 45 (1989) 28–33.
[16] L.K. Low, N. Castagnoli, Jr., in: J.N. Delgado, W.A. Remers (Eds.), Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott, Philadelphia, 1991, pp. 45–121.