

PROPAFENONE PHARMACOKINETICS: GLC-ECD ANALYSIS; METABOLIC
INDUCTION BY PHENOBARBITAL IN NON-SMOKING AND SMOKING HEALTHY
VOLUNTEERS; PROTEIN BINDING; PHARMACODYNAMICS IN PATIENTS

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Faculty of Pharmaceutical Sciences)
(Division of Pharmaceutics)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
August, 1989

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Date September 11, 1989

ABSTRACT

Propafenone (PF) is a new class I antiarrhythmic agent used to treat supraventricular and ventricular tachyarrhythmias.

This thesis reports: a) an *in vitro* protein binding study of PF in normal and uremic sera; b) a drug-drug interaction study of PF and phenobarbital in healthy human subjects and c) a concentration-response relationship study of PF in patients. In order to conduct these studies, it was necessary to develop a sensitive and accurate assay method for the measurement of PF in biological fluids.

A capillary column electron-capture detection gas-liquid chromatographic (GLC-ECD) assay was developed for the quantitation of PF. The identity of the derivative formed with heptafluorobutyric anhydride was confirmed by GLC-mass spectrometry. The limit of determination of the assay method was 2.5 ng/mL using 1 mL of serum. The GLC-ECD method developed for the quantitation of PF was further modified to measure the major and active metabolite of PF, 5-hydroxy PF.

The serum protein binding of PF was examined and characterized *in vitro* in serum obtained from healthy human subjects using equilibrium dialysis. Two binding sites, one high-affinity, low-capacity and one low-affinity, high-capacity, were apparent. The serum protein binding of PF was found to be concentration-dependent, with PF free fraction increasing from 0.03 to 0.19 as PF concentration increased from 0.25 to 100 $\mu\text{g/mL}$. However, no evidence for significant concentration-dependent changes in binding were observed within the PF concentration range of 0.25-1.5 $\mu\text{g/mL}$, which covered a major portion of the therapeutic concentration range (0.5-2 $\mu\text{g/mL}$).

In pooled uremic serum, the PF free fraction was approximately 50% of that of the PF free fraction in normal serum throughout the concentration range studied (1-5 $\mu\text{g/mL}$). In serum from patients with chronic renal failure, the increase in PF binding ratio was positively and significantly correlated with the increase in serum α_1 -acid glycoprotein (AAG) concentration, suggesting that AAG is an important binding protein for PF in serum.

The effect of enzyme induction on the pharmacokinetics of PF and its major and active metabolite, 5-hydroxy PF, was studied in eight healthy non-smoking and eight healthy heavy cigarette smoking Caucasian males (age 20-45 y). Each subject received a single oral dose of PF (300 mg) on two occasions, separated by 23 days of phenobarbital treatment (100 mg daily at bedtime). Except for two smokers who were 'slow' metabolizers, all non-smoking and smoking subjects were 'rapid' metabolizers (intrinsic clearance, $\text{CL}_{\text{int}} > 0.5 \text{ L/min}$). Since there was great intersubject variability in most kinetic parameters calculated, each subject served as his own control. Phenobarbital induced hepatic microsomal enzymes and enhanced the extent of the first-pass metabolism of PF. There was a significant increase in CL_{int} after phenobarbital treatment. The increase in CL_{int} ranged from 10-831% in the non-smokers and 23-450% in the smokers, resulting in a substantial decrease in the systemic availability, as measured by a reduction in PF peak concentration (C_{max}) and the area under the serum concentration-time curve (AUC). The decrease in C_{max} ranged from 0-87% in the non-smokers and 8-85% in the smokers while the decrease in AUC ranged from 10-89% in the non-smokers and 19-82% in the smokers. Except for two smoking subjects, the percent decrease in serum AUC was similar to the percent decrease in salivary AUC noted after enzyme induction in the

non-smoking and the smoking subjects.

Phenobarbital treatment did not lead to increases in the serum concentration, C_{\max} or the AUC of 5-hydroxy PF. Furthermore, there was no observed increase in the renal excretion of the conjugates of either 5-hydroxy PF or 5-hydroxy-4-methoxy PF, a subsequent metabolite of 5-hydroxy PF.

Twenty-three days of phenobarbital treatment did not cause any change in PF free fraction or serum AAG concentration in the non-smoking and the smoking subjects.

A wide range in the extent of metabolic induction of PF by phenobarbital, expressed as percent decrease in AUC, was observed in the non-smokers and the smokers, and in 'rapid' and 'slow' metabolizers. Enzyme induction did not convert 'slow' metabolizers of PF to 'rapid' metabolizers. Furthermore, the extent of metabolic induction of PF by phenobarbital was independent of the individual's polymorphic phenotype, serum phenobarbital concentration or the apparent initial ability of the individual's liver to metabolize drugs, *i.e.*, $CL_{\text{int control}}$.

When compared to the non-smokers, heavy cigarette smokers had a significantly larger CL_{int} , a lower C_{\max} and a smaller AUC. While smoking did appear to increase the clearance of PF, it is difficult to conclude that smoking induced the metabolism of PF, due to the small sample size and the lack of comparison of smokers (serving as their own experimental control) under a nonsmoking circumstance.

The concentration-response relationship of PF was studied in 10 patients (age 30-71 y) receiving PF (mean daily oral dose = 650 mg) for treatment of supraventricular arrhythmias. The QRS width measured from signal-averaged electrocardiograms (150 beats) was used as an indicator of

the antiarrhythmic response of PF. The correlation between QRS width and several parameters such as PF serum concentration, 5-hydroxy PF serum concentration and serum AAG concentration was examined. Each of these parameters seemed to contribute to or influence the overall pharmacological effect of PF. It was possible to predict QRS width from the values of these parameters using an equation developed from multiple stepwise regression. The equation was described as $Y = 0.5X_1 + 4.5X_2 + 347X_3 + 79$ where Y was QRS width, X_1 was log PF serum concentration, X_2 was log 5-hydroxy PF serum concentration and X_3 was the reciprocal of serum AAG concentration.

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LIST OF ABBREVIATIONS

AAG	α_1 -acid glycoprotein
AUC	area under the concentration <i>versus</i> time curve
AUC_0^t	area under the concentration <i>versus</i> time curve from time zero to time t
AUC_0^∞	area under the concentration <i>versus</i> time curve from time zero to infinity
AUMC	area under the first moment of the concentration <i>versus</i> time curve
AV	atrioventricular
β	apparent elimination rate constant describing the terminal portion of the serum drug concentration <i>versus</i> time curve
C_{max}	peak concentration of drug in serum
C_p	drug concentration in serum at time t
C_{pss}	drug serum concentration at steady-state
CL	clearance
CL_{cr}	creatinine clearance
CL_{int}	intrinsic clearance
CL_o	oral clearance
C.V.	coefficient of variation
D	dose
E	extraction efficiency
ECD	electron-capture detector
ECG	electrocardiogram
EI	electron-impact
F	systemic availability

GLC	gas-liquid chromatography
GLC-ECD	gas-liquid chromatography-electron-capture detection
GLC-MS	gas-liquid chromatography-mass spectrometry
GI	gastrointestinal
HFB	heptafluorobutryl
HFBA	heptafluorobutyric anhydride
HP	Hewlett-Packard
HPLC	high-performance liquid chromatography
I.D.	internal diameter
I.S.	internal standard
i.v.	intravenous
IU	international unit
k_a	absorption rate constant
MRD	maximum rate of depolarization
M.W.	molecular weight
NDPP	N-depropyl propafenone
NICI	negative-ion chemical-ionization
PAH	polycyclic aromatic hydrocarbons
PF	propafenone
PFPA	pentafluoropropionic anhydride
PICI	positive-ion chemical-ionization
PTFE	polytetrafluoroethylene
PVCs	premature ventricular contractions
r	correlation coefficient
RID	radial immunodiffusion
rpm	revolutions per minute
S	statistically significant

SAECGs	signal-averaged electrocardiograms
s.d.	standard deviation
SGOT	serum glutamic oxaloacetic transaminase
t	time
$t_{1/2}$	half-life
t_{\max}	time to reach peak concentration
τ	dosing interval
TEA	triethylamine
TFAA	trifluoroacetic anhydride
TMA	trimethylamine
UHP	ultra high purity
UV	ultraviolet
V_c	volume of distribution of the central compartment
V_d	volume of distribution
$V_{d\text{area}}$	volume of distribution according to area or clearance method
V_{dss}	volume of distribution at steady-state
WPW	Wolff-Parkinson-White

ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. James Axelson for his supervision, guidance and financial support. Special gratitude for his valuable advice, unfailing support and unceasing encouragement throughout the study.

I would also like to thank my committee members Drs. Jim Orr, Charles Kerr, Keith McErlane and John Sinclair for their encouragement and support throughout the Ph.D. program.

Thanks to Drs. C. Kerr and J. Yeung for their supervision of study subjects. Thanks go to Dr. F. Abbott for his help with the use of the GC-MS and his capable assistance in the interpretation of GC-MS data. I would also like to thank R. Burton for his extensive help with the GC-MS.

My appreciation to B. McErlane for her assistance in collecting blood samples, N. Wong for her help in collecting uremic blood samples and S. Tse for her help with the measurement of the binding proteins.

Thanks to my fellow graduate students Sun Dong Yoo, Jing Wang, K. Yeleswaram and Rajesh Mahey and my friends Paul Molund, Roland Burton and Jacqueline Walisser for their inspiration and friendship. Special thanks to Wayne Riggs, Matthew Wright, George Tonn and Sue Panesar for their constructive criticism in the writing of the thesis and their constant support and encouragement.

This project was supported by the British Columbia Heart Foundation, the Kidney Foundation of Canada and Knoll Pharmaceuticals Canada Inc. Finally, I would like to gratefully acknowledge the receipt of Traineeships from the University of British Columbia, the British Columbia Heart Foundation and the Canadian Heart Foundation.

This thesis is dedicated
to
my wonderful parents, whose unselfish and boundless love,
continuous support and encouragement
have carried me through my Ph.D. program,
to
my dear sister and brother
and to
the memory of my grandfather and grandmother

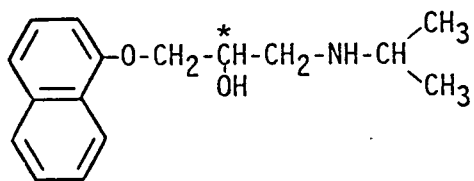
1. INTRODUCTION

1.1 General Background

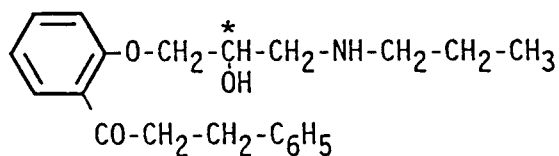
Propafenone (PF) is a potent, generally well-tolerated and orally effective new antiarrhythmic agent with demonstrated effectiveness against a variety of cardiac arrhythmias [Siddoway *et al.*, 1984a; Harron and Brogden, 1987; Schlepper, 1987]. Propafenone was synthesized in 1970 (Knoll Pharmaceuticals, Germany) and has been available in Europe since 1977 as Rytmonorm^R and in Canada since 1987 as Rhythmol^R.

Propafenone is a basic compound ($pK_a = 9.0$) and is slightly soluble in water (solubility <1%). Propafenone hydrochloride appears as colorless crystals or a white crystalline powder with a bitter taste.

Propafenone {2-(2'-hydroxy-3'-propylamino-propoxy)- ω -phenyl-propiophenone} contains an asymmetric carbon and is used as a racemate containing the R and S-enantiomers (Figure 1). Propafenone is a weak β -adrenoceptor blocker and is structurally similar to other β -blockers such as propranolol (Figure 1A), alprenolol, metoprolol, oxprenolol and practolol (Figure 1B). The substituent at R_1 is usually an isopropyl group but in PF it is n-propyl. The structure-activity relationship of these β -blockers has been examined. The size and position of the other substituents on the benzene ring (*i.e.*, R_2 and R_3) appear to be important in determining the cardioselective potency of β -adrenoceptor blockers. Compounds with a substituent at R_3 (para-position) have been found to be more cardioselective than those with a substituent at R_2 (ortho position) [Bagwell and Vaughan Williams, 1973; Vaughan Williams *et al.*, 1973].

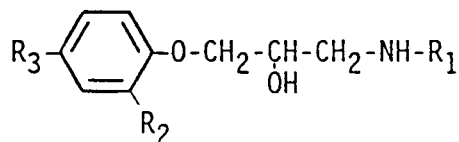


propranolol



propafenone

Figure 1A. Chemical structures of propafenone and propranolol (* is the asymmetric carbon that gives rise to stereoisomerism).



Compound	R ₁	R ₂	R ₃
Alprenolol	-CH(CH ₃) ₂	-CH ₂ -CH=CH ₂	-H
Metoprolol	-CH(CH ₃) ₂	-H	-CH ₂ -CH ₂ -OCH ₃
Oxprenolol	-CH(CH ₃) ₂	-OCH ₂ -CH=CH ₂	-H
Propafenone	-CH ₂ -CH ₂ -CH ₃	-CO-CH ₂ -CH ₂ -C ₆ H ₅	-H
Practolol	-CH(CH ₃) ₂	-H	-NH-CO-CH ₃

Figure 1B. Chemical structures of propafenone and other β -adrenoceptor blockers.

1.2 Therapeutic and Clinical Effects of Propafenone

Clinical studies have shown that PF is effective in treating and suppressing chronic recurrent supraventricular and ventricular tachyarrhythmias and ectopic beats [Waleffe *et al.*, 1981; Chilson *et al.*, 1982; Connolly *et al.*, 1983a; Hodges *et al.*, 1984; Podrid *et al.*, 1984; Rabkin *et al.*, 1984; Salerno *et al.*, 1984; Siddoway *et al.*, 1984b; Dinh *et al.*, 1988]. Propafenone has also been shown to be effective in the short and long-term treatment of supraventricular tachycardias associated with the Wolff-Parkinson-White (WPW) syndrome [Rudolf *et al.*, 1979; Breithardt *et al.*, 1984] and in the prevention of recurrent atrial fibrillation [Kerr *et al.*, 1988].

1.3 Comparison of Propafenone to Placebo and Other Antiarrhythmic Agents in the Treatment of Arrhythmias

Propafenone was shown to be an effective antiarrhythmic agent with an acceptable frequency of adverse effects when used in patients with severe ventricular arrhythmias who were refractory to other antiarrhythmic agents or who had significant intolerance to these drugs [Podrid and Lown, 1984; Rabkin *et al.*, 1984].

Propafenone was also demonstrated to be superior to placebo in suppressing ventricular ectopic activity [Salerno *et al.*, 1982, Hodges *et al.*, 1984; Salerno *et al.*, 1984; Soyza *et al.*, 1984; Naccarella *et al.*, 1985]. In several double-blind crossover studies, PF appeared to have comparable or greater efficacy than other class I antiarrhythmic agents.

For example, PF showed comparable efficacy to quinidine for the control of ventricular arrhythmias in ambulatory patients with diverse forms of heart diseases [Dinh *et al.*, 1985]. In patients with frequent ventricular arrhythmias who had not responded to treatment with procainamide (50 mg/kg/day) or quinidine (1200 mg/day), PF exhibited greater efficacy over either drug in the suppression of premature ventricular contractions (PVCs). When combined with either procainamide or quinidine, lower doses of PF could be used effectively than with PF alone [Klein *et al.*, 1987]. Propafenone also had greater efficacy over disopyramide in the treatment of frequent and complex PVCs [Naccarella *et al.*, 1982; Naccarella *et al.*, 1985]. Besides class I agents, PF also showed similar efficacy to amiodarone in the short-term treatment of chronic, isolated or repetitive ventricular extrasystoles [Fauchier *et al.*, 1986].

1.4 Antiarrhythmic Drug Classification of Propafenone

The classification of antiarrhythmic agents into four classes according to their individual electrophysiological and pharmacological actions was first proposed by Vaughan Williams in 1970 and was later updated by Harrison in 1983 (Table 1A). Class I agents had further been subdivided into three groups (Table 1B), based on the kinetics (rapidity) of attachment of the drug to, and its detachment from, the sodium channels [Vaughan Williams, 1984] or on their effect on the duration of the action potential [Harrison *et al.*, 1985]. Previous studies indicated that PF exerted classes I, II and IV antiarrhythmic action and it was classified as primarily a class IC agent, with little or no effect on myocardial

Table 1A. Classification of antiarrhythmic agents [Vaughan Williams, 1970; Harrison, 1983].

Class		Mechanism of action	Examples
I	sodium channel blockers	depress fast inward current	see Table 1B
II	β -adrenoceptor blockers	inhibit sympathetic activity	propranolol metoprolol
III	-	prolong repolarization	amiodarone bretylium
IV	calcium channel blockers	depress slow inward current	nifedipine verapamil

Table 1B. Subdivision of class I antiarrhythmic agents [Vaughan Williams, 1984].

Class	Examples	Characteristics of Class
IA	quinidine disopyramide procainamide cibenzoline	a) widens QRS and slows conduction at high concentrations b) prolongs QT interval and lengthens duration of action potential c) lengthens refractory periods
IB	lidocaine mexiletine tocainide phenytoin	a) limit effect on QRS and conduction b) shortens repolarization and QT interval c) elevates fibrillation threshold
IC	flecainide encainide lorcainide indacainide	a) widens QRS and slows conduction at low concentration b) slight effect on repolarization and duration of action potential c) small changes in refractoriness

repolarization [Dukes and Vaughan Williams, 1984].

1.4.1. Class I-Like Action of Propafenone

Propafenone has a strong membrane stabilizing or local anaesthetic effect [Fill *et al.*, 1977; Zeiler *et al.*, 1984]. It reduces automaticity, conductivity and excitability in all cardiac tissues. Propafenone causes a dose-dependent decrease in the maximum rate of depolarization (MRD) and in the overshoot of the action potential in all tissues normally depolarized by fast sodium current. In isolated rabbit proximal and distal Purkinje fibers, the MRD and overshoot of the action potential have been demonstrated to reduce to a greater extent than in the ventricle or atrial tissues [Dukes and Vaughan Williams, 1984]. Although PF inhibits the fast inward current (class I action), it also exhibits actions of other classes and cannot be considered as a selective inhibitor of the sodium channel.

1.4.2. Class II-Like Action of Propafenone

Propafenone contains in its molecular structure, the aryloxy propanolamine structure common to β -blockers. Propafenone exhibits a competitive antagonism of the chronotropic and inotropic effects of the β -agonist isoproterenol (*i.e.*, inhibition of isoproterenol-induced tachycardia and decrease in blood pressure) [Ledda *et al.*, 1981; McLeod *et al.*, 1984]. Furthermore, PF has a selectivity of β_2 -adrenoceptors over β_1 -adrenoceptors; the β_2 -blocking effect is three times stronger than the β_1 -blocking effect on the heart [Dukes and Vaughan Williams, 1984]. In isolated cardiac preparations, the β -adrenoceptor blocking and the membrane stabilizing effects occur in an approximately equal concentration range [Ledda *et al.*, 1981]. In comparison to propranolol, the β -blocking effect

of PF appears to be 2-5% that of propranolol on the basis of isoproterenol sensitivity testing [Muller-Peltzer *et al.*, 1983; McLeod *et al.*, 1984].

1.4.3. Class IV-Like Action of Propafenone

Propafenone has some depressive effects on the calcium channel (class IV action). This is supported by the clinical evidence that PF depresses AV conduction and that it is effective against WPW type arrhythmias [Dukes and Vaughan Williams, 1984]. In patients with the WPW syndrome, PF slows (and, in some cases, completely blocks) the anterograde and retrograde conduction and prolongs the refractory period of the accessory pathway, irrespective of the initial length [Waleffe *et al.*, 1981; Breithardt *et al.*, 1984]. The calcium channel blocking effect of PF is extremely weak, being ~1% that of verapamil or nifedipine [Ledda *et al.*, 1981; Dukes and Vaughan Williams, 1984].

1.5. Electrophysiological Effects of Propafenone and 5-Hydroxy Propafenone

Propafenone decreases the automaticity of the sinus node and significantly prolongs corrected sinus node recovery time [Breithardt *et al.*, 1984]. In clinical studies in patients, heart rate has been found to be either increased [McLeod *et al.*, 1984] or unaffected by therapeutic doses of PF [Breithardt *et al.*, 1984]. In conscious dogs with chronic atrioventricular (AV) block, PF is believed to increase atrial rate by a direct and reflex vagolytic action at plasma concentrations within the therapeutic range (0.5-2 $\mu\text{g/mL}$). However, the bradycardia which occurs at lower PF concentrations (<0.33 $\mu\text{g/mL}$) seems to be due to its membrane

stabilizing activity [Li et al., 1985]. When administered in a dose of 0.5-2 mg/kg, PF initially increases ventricular rate through a reflex response to its hypotensive effect. This increase in ventricular rate is reversed at high doses (4 mg/kg) and ventricular bradycardia results due to its membrane stabilizing and/or its weak β -blocking effects.

Propafenone prolongs the refractory period of normal atria, AV node and ventricular tissue [Connolly et al., 1983a] and elevates the ventricular fibrillation threshold [Breithardt et al., 1984]. Propafenone also suppresses AV nodal conduction, His bundle conduction and intraventricular conduction. Furthermore, it lengthens intracardiac conduction times, including the PA (intra-atrial), AH (AV nodal) and HV (His-Purkinje system) intervals [Connolly et al., 1983a; Breithardt et al., 1984; Feld et al., 1987]. These effects are shown in electrocardiographic measurement as a prolonged PR interval (~16%), a widened QRS complex (~18%) and a prolonged QT interval [Connolly et al., 1983a; Hodges et al., 1984; Salerno et al., 1984]. However, prolongation of the QT interval is minor [Connolly et al., 1983b; Hodges et al., 1984].

The presence of active metabolites found in the plasma of patients receiving chronic oral therapy with encainide or lorcinide has been suggested to account for the electrophysiological differences between orally and intravenously administered drugs [Jackman et al., 1982; Echt et al., 1983]. Unlike lorcinide and encainide, the electrophysiologic effects produced by oral dosing of PF has been found to be qualitatively similar to those produced by intravenous (i.v.) dosing [Connolly et al., 1983a]. This suggests that if there were an active metabolite accumulating during oral therapy of PF, its electrophysiological effects must be qualitatively similar to those of PF [Connolly et al., 1983a]. Studies of

the electrophysiologic effects of 5-hydroxy PF on guinea pig ventricular muscle fibers [Valenzuela *et al.*, 1987] and in canine Purkinje fibers [Thompson *et al.*, 1988] have indicated that the electrophysiological effects of 5-hydroxy PF, a major metabolite of PF, are similar to those described with PF.

1.6 Pharmacokinetics of Propafenone

Studies using deuterium-labeled PF showed that >95% of the administered dose was absorbed from the gastrointestinal (GI) tract after oral administration [Hollmann *et al.*, 1983b]. Peak plasma concentration was reached 2 to 3 h after the dose [Keller *et al.*, 1978; Seipel and Breithardt, 1980; Connolly *et al.*, 1983b; Hollmann *et al.*, 1983a]. After administration of an intravenous dose of PF to normal volunteers, PF distributed rapidly to body tissues and plasma concentrations then declined, with the mean apparent terminal half-life of PF in plasma being approximately 5 h [Connolly *et al.*, 1984].

Propafenone was shown to bind extensively yet variably to plasma protein. The binding was found to be approximately 97% at PF plasma concentrations up to 1.6 $\mu\text{g/mL}$, and decreasing slightly at higher PF concentrations (94% at 7 $\mu\text{g/mL}$ and 88% at 36 $\mu\text{g/mL}$) [Hollmann, 1983b]. The volume of distribution describing the central compartment (V_c) was approximately 0.7-1.1 L/kg in normal volunteers, after the administration of an i.v. bolus of PF. The apparent half-life for distribution of drug from the central compartment was approximately 4 minutes. The steady state volume of distribution (V_{dss}) was approximately 1.9-3 L/kg in healthy

subjects [Seipel and Breithardt, 1980; Hollmann *et al.*, 1983a]. This large distribution volume, which was far greater than any actual body space, seemed to indicate a considerable binding of PF in peripheral tissues. At autopsy, PF was found in the lung at a concentration about ten-fold higher than that in heart muscle or liver and twenty-fold higher than that in skeletal muscle and kidney [Seipel and Breithardt, 1980]. The metabolite, 5-hydroxy PF, was found in the heart at a concentration comparable to that of PF [Latini *et al.*, 1987].

While PF was shown to be completely absorbed [Hollmann *et al.*, 1983b], its bioavailability was found to be 5 and 12% for the 150 and 300 mg film-coated tablets, respectively, due to the extensive presystemic elimination of PF after oral dosing. Hollmann *et al.* [1983a] found a non-linear, disproportionate increase in the area under the concentration versus time curve (AUC) of PF with increasing dose. In their study, the systemic bioavailability of PF increased from 4.8 to 12.1% when the dose of PF was increased from a 150 to a 300 mg tablet. The bioavailability of PF was further increased to 23.5% when the 300 mg dosage was administered as an oral solution. Connolly *et al.* [1983b] studied the relationship between dose and steady-state mean plasma concentration of PF during a dose-ranging study in patients with arrhythmia. They found that as the dose of PF increased three-fold from 300 to 900 mg, there was a ten-fold increase in the steady-state mean plasma concentration. This non-linear relationship between AUC and dose or steady-state plasma concentration, also seen with lorcinide kinetics [Klotz *et al.*, 1978], was suggested to be a result of saturation of first-pass metabolism. The saturable first-pass metabolism could lead to a rapid increase in PF plasma concentration with increasing dosage beyond the 'saturation' point [Siddoway *et al.*, 1984a].

Studies of single-dose elimination kinetics indicated that the apparent elimination half-life of PF was relatively short, being 4.6 h (range, 2.3-9.5 h; 300 mg dose) in healthy volunteers [Hollmann *et al.*, 1983a] and 3.6 h (range, 1.8-4.3 h; 900 mg dose) in patients with cardiac dysrhythmias [Keller *et al.*, 1978]. Studies of steady-state elimination kinetics of PF in patients requiring antiarrhythmic therapy showed longer elimination half-lives and greater interindividual variability. Connolly *et al.* [1983b] reported a wide range of elimination half-life of 2.4 to 11.8 h. Similarly, Salerno *et al.* [1984] reported a range of 1.8 to 17.2 h while Siddoway *et al.* [1983] reported a range of 1.8 to 32.3 h. Connolly *et al.* [1983b] found that the large interindividual variability in the elimination half-life observed in his patients could not be explained by clinical factors such as age, liver disease, heart failure or the presence of concomitant drug therapy that could interfere with the elimination of a highly metabolized drug. He suggested that, like procainamide [Reidenberg *et al.*, 1975] and encainide [Roden *et al.*, 1980], individual differences in drug metabolism may cause the variation seen in the elimination half-life of PF. Another explanation for the longer elimination half-life of PF during chronic oral dosing was a decrease in the apparent clearance of the drug [Connolly *et al.*, 1984].

Siddoway *et al.* [1983] reported that PF undergoes metabolism *via* a polymorphic oxidative pathway which correlated with the debrisoquine oxidative phenotype. The population was divided into 'rapid' and 'slow' metabolizers of debrisoquine on the basis of the urinary ratio of debrisoquine to its 4-hydroxy metabolite after a single oral dose. The inhibition of debrisoquine 4-hydroxylation by PF was further demonstrated both *in vivo* and in a human liver microsomal system *in vitro* [Siddoway *et*

a1., 1987]. This suggested that PF was metabolized *via* the same cytochrome P-450 systems which was responsible for the oxidation of debrisoquine [Siddoway *et al.*, 1987]. In pharmacokinetic and pharmacodynamic studies of PF in patients with arrhythmia [Siddoway *et al.*, 1983; Siddoway *et al.*, 1987], 'slow' metabolizers of debrisoquine appeared to have a long PF apparent elimination half-life (16.8 ± 0.6 h or 17.2 ± 8.0 h), high plasma concentrations at steady-state and after single dosing (2.16 ± 0.49 ng/mL/mg dose or 2.5 ± 0.5 ng/mL/mg daily dosage), low oral clearance (0.26 ± 0.05 mL/min), proportional dose-concentration relationship and an absence of detectable 5-hydroxy PF in plasma. On the other hand, 'rapid' metabolizers were characterized by a relatively short apparent elimination half-life (4.7 ± 1.3 h or 5.5 ± 2.1 h), low plasma concentrations at steady-state and after single dosing (0.81 ± 0.51 ng/mL/mg dose or 1.1 ± 0.6 ng/mL/mg daily dosage), high oral clearance (1.12 ± 1.24 L/min), disproportional dose-concentration relationship and detectable quantities of 5-hydroxy PF in plasma.

1.7 Metabolism of Propafenone

The metabolic pathway of PF in man is illustrated in Figure 2. Following oral administration of PF, the drug is subject to extensive first-pass metabolism, with less than 1% of the administered dose excreted unchanged in the urine or in the feces [Seipel and Breithardt, 1980; Hollmann *et al.*, 1983b; Hege *et al.*, 1984a]. Propafenone is metabolized to 5-hydroxy PF and hydroxy-methoxy PF *via* aromatic hydroxylation, a major metabolic pathway of PF [Hollmann *et al.*, 1983b]. The mechanism is

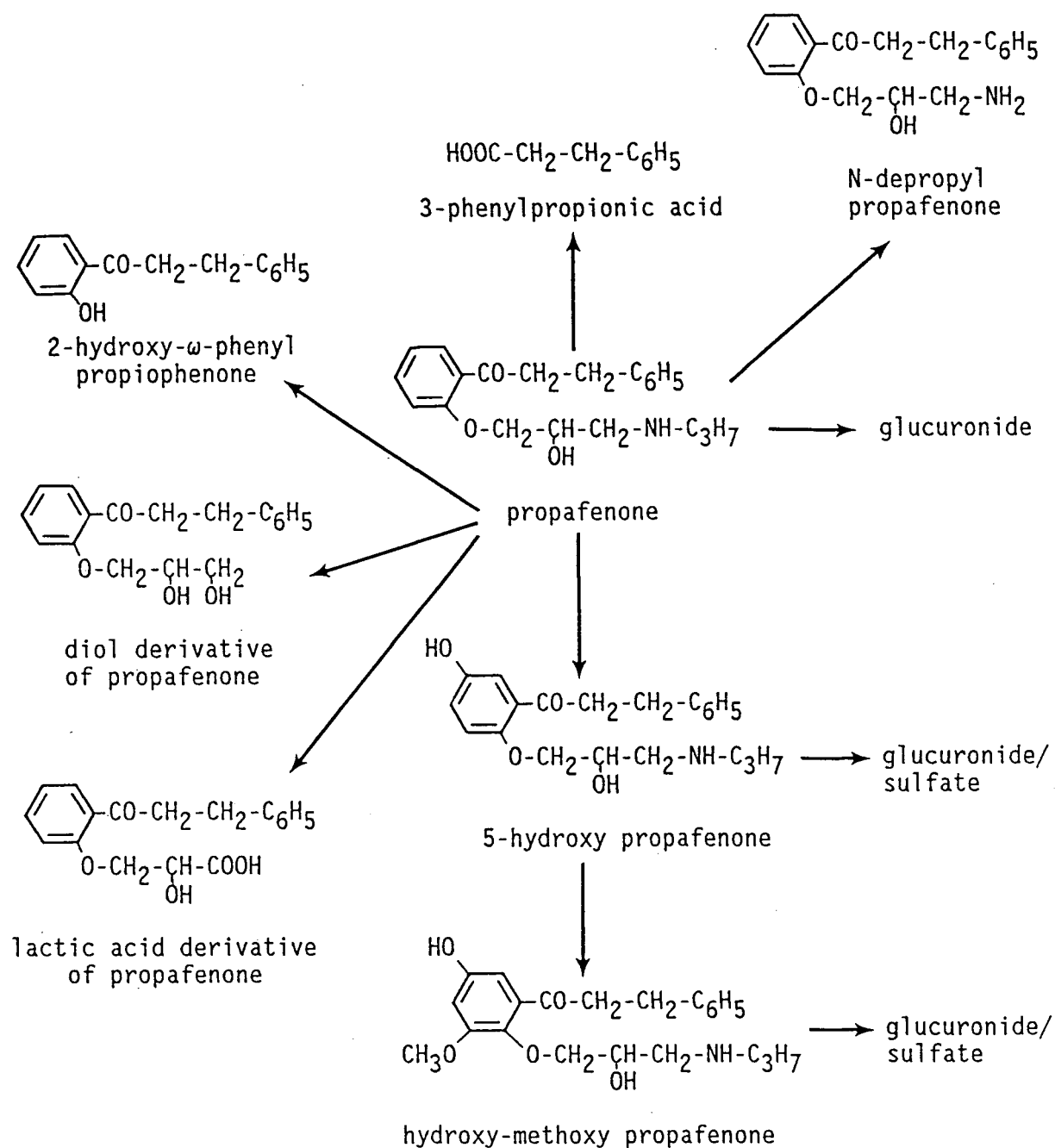


Figure 2. Metabolic pathways of propafenone in man [Hege et al., 1984].

suggested to be similar to that of propranolol where an intermediate arene oxide is formed, followed by opening of the oxiran ring to the hydroxylated product in the para position to the alkoxy oxygen [Walle *et al.*, 1982]. In addition to oxidative metabolism, PF, 5-hydroxy PF and hydroxy-methoxy PF also conjugate with glucuronic and sulphuric acids. These conjugates are the primary form in which PF and its oxidative metabolites are recovered in bile, urine and feces [Hollmann *et al.*, 1983b; Hege *et al.*, 1984a]. The glucuronide and sulfate conjugates of 5-hydroxy PF and hydroxy-methoxy PF and PF glucuronide appear in a ratio of 3:2:1 in plasma and urine and in a ratio of 2:1:1 in bile [Hege *et al.*, 1984a]. There is individual variation in the proportion of the excretion products in the urine [Hege *et al.*, 1984a]. The primary route of excretion of the metabolites is *via* the feces. Most of the hydroxylated metabolites in the feces are found to be conjugates. Although the GI tract contains microfloral β -glucuronidase that is capable of hydrolyzing the conjugates and releasing the free drug which can then be reabsorbed [Levine, 1978], it is believed that enzymatic cleavage of PF may be hindered in the feces and there is no evidence of enterohepatic circulation [Hege *et al.*, 1984a].

Other monohydroxylated metabolites of PF have not yet been identified [Hege *et al.*, 1984a]. Di-hydroxylated products (*via* corresponding arene oxides and dihydrodiol intermediates) have so far been discovered only rarely as they are rapidly converted to hydroxy-methoxy derivatives by catechol-o-methyl transferase [Hege *et al.*, 1984a]. Other minor metabolic pathways of PF identified in man include N-dealkylation followed by oxidative deamination to form a glycol and a lactic acid derivative (this pathway is common to many endogenous and exogenous amines and most β -blockers), C-C splitting to yield a relatively large amount of

3-phenylpropionic acid and cleavage of the ether group to yield 2-hydroxy- ω -phenyl propiophenone. The metabolite 3-phenylpropionic acid is found mainly in the feces and, while it may be a metabolic product, it may also be a breakdown product of the original metabolite (unidentified, speculation only) during drug analysis [Hege *et al.*, 1984a]. Another metabolite, N-depropyl PF (NDPP), formed by N-dealkylation, has been qualitatively identified [Latini *et al.*, 1988].

The major metabolite of PF, 5-hydroxy PF, has been found to be pharmacologically active in animal models [Philipsborn *et al.*, 1984; Valenzuela *et al.*, 1987; Thompson *et al.*, 1988]. In isolated tissues (guinea-pig atria, rat aortic strips), 5-hydroxy PF has been demonstrated to have a greater negative inotropic and calcium channel blocking effect but a weaker β -adrenoceptor blocking and local anesthetic effect than PF [Philipsborn *et al.*, 1984]. In canine Purkinje fibers, the electrophysiological effects of NDPP appears to be similar to but less active than those of PF and 5-hydroxy PF [Thompson *et al.*, 1988]. In *in vivo* studies (dog and rat), 5-hydroxy PF has exhibited a more potent antiarrhythmic effect and a lower β -blocking effect than PF [Philipsborn *et al.*, 1984]. Two metabolites, 5-hydroxy PF and NDPP, have been found to accumulate in the plasma of patients during chronic oral PF therapy for frequent ventricular ectopy, although the mechanism is unclear [Kates *et al.*, 1985]. The pharmacological and antiarrhythmic effects of NDPP have yet to be examined.

1.8 Adverse Effects of Propafenone

The major adverse effects of PF include cardiac, neurologic and

gastrointestinal effects. Other effects such as leukopenia, transient cholestatic hepatitis and cutaneous rash have been reported but are rare [Seipel and Breithardt, 1980].

1.8.1 Cardiac Adverse Effects

Propafenone seems to prolong certain ECG parameters without causing symptoms or significant bradyarrhythmia [de Soyza *et al.*, 1984]. All antiarrhythmic agents have some capacity for worsening arrhythmias [Velebit *et al.*, 1982]. Arrhythmogenicity of PF, such as worsening of ventricular ectopy or aggravation of ventricular arrhythmia, has been reported [Seipel and Breithardt, 1980; Connolly *et al.*, 1983a; Nathan *et al.*, 1984; Podrid and Lown, 1984]. Other unwanted cardiac effects observed include depression of sinus node function, development of conduction abnormalities, such as SA block, first degree and second degree AV block and bundle branch block and exacerbation of congestive heart failure [Hodges *et al.*, 1984; Podrid and Lown, 1984; Salerno *et al.*, 1984].

Propafenone has been demonstrated to exert a significant negative inotropic effect on the heart [Karagueuzian, 1984; Salerno *et al.*, 1984] and depress left ventricular function in patients with baseline myocardial dysfunction [Baker *et al.*, 1984]. The magnitude of the negative inotropic effect of PF appears to be only half that of disopyramide in man [Wester and Mouselimis, 1982]. In patients with myocardial impairment (ejection fraction <50%), PF therapy have been shown to further reduce left ventricular function. However, in patients with normal ejection fractions (50-70%), this drug has no effect on left ventricular function [Podrid and Lown, 1984; Podrid *et al.*, 1984].

1.8.2 Neurologic Adverse Effects

Central nervous system adverse effects of PF include visual blurring, headache, lightheadedness, dizziness, vertigo and paresthesias [Rabkin *et al.*, 1984; Siddoway *et al.*, 1987].

1.8.3 Gastrointestinal Adverse Effects

Gastrointestinal adverse effects of PF include metallic taste, dry mouth, epigastric discomfort, nausea, vomiting and constipation [Connolly *et al.*, 1983b; Rabkin *et al.*, 1984].

1.9 Serum Concentration-Response Relationship of Propafenone and Clinical Monitoring of Drug Effect in Patients

The concentration-response relationship for arrhythmia suppression by PF has shown remarkable intersubject variability and, unfortunately, has yet to be established. Connolly *et al.* [1983b] have observed a wide range of 'therapeutic' plasma concentration of PF (64-1044 ng/mL) in their patients. Siddoway *et al.* [1983] have also noted a wide range of mean plasma concentrations of PF, ranging from 143 to 1992 ng/mL, at an apparently effective PF dosage. Similarly, Salerno *et al.* [1984] have reported that the minimum effective trough plasma concentrations of PF ranged from 91 to 3271 ng/mL in their patients. Connolly *et al.* [1983b] have found an approximately log-linear relationship between steady-state plasma concentration and antiarrhythmic response at intermediate concentrations (100-1000 ng/mL) in individual patients undergoing a short-term PF oral therapy (no antiarrhythmic response was seen at concentrations

less than 100 ng/mL).

The therapeutic concentration of PF, defined as the concentration at which 90% suppression of PVCs occurred, has been reported to be within 0.5 and 2 $\mu\text{g/mL}$ [Seipel and Breithardt, 1980]. However, the ability to suppress PVCs does not necessarily correlate with antiarrhythmic efficacy in the treatment of more serious arrhythmias with this drug [Connolly *et al.*, 1983a].

The adverse effects of PF have been suggested to be dose-dependent, although no clear-cut relationship has been demonstrated between plasma concentration and adverse effects [Siddoway *et al.*, 1984]. Therefore, adjustment of the dose can sometimes show persistent effectiveness with low side effects. Siddoway *et al.* [1984] have reported that patients with neurologic side effects have plasma concentrations greater than 1100 ng/mL. Moreover, the incidence of central nervous system side effects appears to be significantly higher in 'slow' metabolizers than in 'fast' metabolizers (67% versus 14%).

The non-linear drug accumulation, polymorphic metabolism, interindividual variability in elimination half-life, steady-state mean plasma concentration and therapeutic plasma concentration of PF (parameters that affect the duration of antiarrhythmic response during long term therapy), indicate a need for individual therapy and control [Connolly *et al.*, 1983b]. Propafenone prolongs the PR interval and QRS duration at therapeutic doses and a significant correlation has been observed between plasma PF concentration and prolongation of the PR interval [Keller *et al.*, 1978; Meyer-Estorf *et al.*, 1978; Meyer-Estorf *et al.*, 1980; Siddoway *et al.*, 1983]. The correlation between plasma PF concentration and prolongation of QRS duration is not clear. Siddoway *et al.* [1987] have

observed a correlation between PF plasma concentration and QRS duration when they excluded the 'slow' metabolizers from their patients. Because of the excellent relationship between PF induced prolongation of PR interval (or QRS duration) and the percent suppression of PVCs, the extent of the increase in the length of the PR interval (or QRS duration) can be used as an index of clinical efficacy [Hodges *et al.*, 1984; Salerno *et al.*, 1984]. To guarantee safety of treatment, the PR and/or QRS intervals should not be prolonged by more than 20% [Naccarella *et al.*, 1982]. Excessive prolongation of the QT interval is associated with arrhythmia aggravation for class I drugs, such as quinidine and disopyramide. However, PF appears to have little effect on the QT interval [Hodges *et al.*, 1984; Salerno *et al.*, 1984] and in patients with arrhythmia exacerbation, excessive prolongation of QT interval has not been observed [Connolly *et al.*, 1983b; Podrid *et al.*, 1984; Buss *et al.*, 1985].

Propafenone is believed to increase the ventricular rate at low concentrations and decrease the ventricular rate at high concentrations. To minimize the risk of ventricular bradycardia, special care and caution should be taken for clinical use of PF when the drug is administered to patients with congestive heart failure [Podrid and Lown, 1984] or to patients with evidence of serious conduction system disease, particularly if the drug is administered by i.v. injection [Connolly *et al.*, 1983b; Podrid and Lown, 1984; Li *et al.*, 1985]. Careful observation of the patient, including ECG monitoring for AV and intraventricular conduction abnormalities, is advisable [de Soyza *et al.*, 1984].

1.10 Dose and Dosage Forms

Propafenone is available in two dosage forms: film-coated tablets containing 150 mg or 300 mg of PF hydrochloride and intravenous injection ampoules containing 70 mg of PF hydrochloride and 1.076 g of dextrose in 20 mL of water.

A single oral dose of 200 or 300 mg or a single i.v. dose of 1 mg/kg of PF has been demonstrated to be well-tolerated and effective in the reduction (oral) or complete repression (i.v.) of extrasystoles or restoration of normal sinus rhythm [Koch, 1977; de Soyza *et al.*, 1984]. Occasionally therapeutic effects are obtained with a single i.v. dose of 0.5 mg/Kg. In arrhythmias, the effective and tolerated i.v. dose range is from 70 to 140 mg [Beck *et al.*, 1975] or 1.5 to 2 mg/Kg depending on the severity of the arrhythmia. The injection should be given slowly. If a second injection is given, it should be 90 to 120 min after the first injection [Bachour and Hochrein, 1977]. In severe cases, PF has been given as an infusion over a period of up to 3 h; occasionally over several days [Bachour and Hochrein, 1977]. Six to eight ampoules per day are generally sufficient for long term infusion. Intravenous administration has often been followed immediately by the oral formulation. The most commonly effective dose of PF for PVCs is 300 mg every 8 h or 900 mg per day, although some patients respond to smaller doses [Hodges *et al.*, 1984].

1.11 Propafenone Drug Interactions

Propafenone reduces the clearance and increases the mean steady-state

plasma concentration of warfarin. The significant increase in prothrombin time indicates an enhanced anticoagulant effect and a requirement for dose adjustment [Kates *et al.*, 1987].

Like quinidine and verapamil [Leahey *et al.*, 1980; Klein *et al.*, 1982], PF also significantly increases plasma digoxin concentration [Belz *et al.*, 1983; Hodges *et al.*, 1984; Salerno *et al.*, 1984] but to a lesser degree than both drugs [Belz *et al.*, 1983]. The mechanism of the increase in plasma digoxin concentration by PF is unknown. Since elevated plasma digoxin concentration may increase the risk of glycoside toxicity, careful monitoring of patients is recommended and adjustment of digoxin dosage may be necessary [Belz *et al.*, 1983; Salerno *et al.*, 1984].

Lidocaine diminishes the prolongation in atrial and ventricular refractoriness produced by PF alone. Lidocaine also produces mild additive negative inotropic effects that may be of hemodynamic significance, especially in patients with ventricular dysfunction [Feld *et al.*, 1987].

In a single dose study in healthy subjects, PF has been demonstrated to cause a two-fold decrease in the oral clearance of metoprolol and increased the duration of β -adrenoceptor blocking activity, as measured by reduction of exercise-induced tachycardia. When administered to patients with cardiovascular disease treated with metoprolol, PF increases the steady-state levels of metoprolol two to five fold. The mechanism is speculated to be competitive inhibition of metoprolol metabolism by PF. It may be necessary to reduce the dose of metoprolol when it is co-administered with PF [Wagner *et al.*, 1987]. On the other hand, the kinetics of PF during chronic therapy seem to be unaffected by coadministration of metoprolol.

1.12 Propafenone-Food Interaction

Food intake has been shown to markedly influence the presystemic metabolism of certain drugs such as propranolol [Melander *et al.*, 1977], metoprolol [Melander *et al.*, 1977], labetalol [Daneshmend and Roberts, 1982] and dicyrazine [Liedholm *et al.*, 1985], resulting in greater bioavailability.

Food intake had been found to have similar effect on the bioavailability of PF [Axelson *et al.*, 1987]. The pharmacokinetics of PF were examined and compared in 24 healthy volunteers (four of the twenty-four subjects were identified as 'slow' metabolizers) in a fasted state and with a standard breakfast. With food, the maximum plasma PF concentration was reached earlier and was significantly increased. When data from 'slow' metabolizers were excluded, there was an average increase of 147% in the AUC following the standard breakfast. Food intake did not affect the bioavailability of PF in 'slow' metabolizers or in subjects with a low intrinsic clearance (CL_{int}). There was a significant correlation ($r = 0.946$, $p < 0.05$) between $[(AUC_{fed} - AUC_{fasted})/AUC_{fasted}]$ and CL_{int} fasted. The authors concluded that patients should be advised to take PF in a constant relationship to food to assure consistent bioavailability [Axelson *et al.*, 1987]. The probable mechanism(s) of the food effect include food induced transient increase in hepatic blood flow rate, changes in the hepatic drug delivery rate, alterations in plasma protein binding, direct inhibition of drug metabolising enzymes by food components or their metabolites, shunting of blood past the liver and/or elimination of the 'threshold dose' effect [Melander *et al.*, 1988].

1.13 Rationale

1.13.1 Rationale for Development of a GLC-ECD Method for Quantitative Analysis of Propafenone

The analytical methods available for the quantitation of PF and their limit of determination are listed in Table 2. Prior to the initiation of the present investigation, several high performance liquid chromatographic (HPLC) methods [Brode, 1982; Brode *et al.*, 1982; Harapat and Kates, 1982; Kannan *et al.*, 1983; Brode *et al.*, 1984] and a gas-liquid chromatographic (GLC) method using a packed column and electron-capture detection (ECD) [Marchesini *et al.*, 1982] were published for the analysis of PF. The HPLC techniques [Brode, 1982; Brode *et al.*, 1982; Harapat and Kates, 1982; Kannan *et al.*, 1983] required a relatively large sample volume (~1-5 mL) and the limit of determination was ~5-20 ng/mL. Unfortunately, these methods lacked the necessary sensitivity to measure trace levels of drug using small sample volumes such as are encountered during single dose kinetic studies and, as well, in protein binding studies. In 1984, Brode *et al.* published an HPLC method using fluorescence detection which displayed a limit of determination for PF as low as 1 ng/mL in 1 mL of human plasma. Further, a highly sensitive gas-liquid chromatography-mass spectrometric (GLC-MS) method for the quantitation of PF in plasma, with a limit of determination as low as 1 ng/mL in 0.5 mL of human plasma, was also published [Higuchi *et al.*, 1985]. The GLC method [Marchesini *et al.*, 1982] using packed-column technology showed greater sensitivity than the early HPLC methods [Brode, 1982; Brode *et al.*, 1982; Harapat and Kates, 1982; Kannan *et al.*, 1983] and permitted the determination of 10 ng/mL of PF. The obtainable sensitivity in modern gas chromatography has been

Table 2. Analytical methods for propafenone measurement.

Author	Analytical method	Limit of determination
Brode, 1982	HPLC	2.5 ng/mL /2 mL plasma
Brode <i>et al.</i> , 1982	HPLC	5 ng/mL /2 mL plasma
Harapat and Kates, 1982	HPLC	5 ng/mL
Marchesini <i>et al.</i> , 1982	GLC	10 ng/mL
Kannan <i>et al.</i> , 1983	HPLC	50 ng/mL
Brode <i>et al.</i> , 1984	HPLC	1 ng/mL /1 mL plasma
Latini <i>et al.</i> , 1988	HPLC	30 ng/mL

HPLC high-performance liquid chromatography
 GLC gas-liquid chromatography

improved tremendously through the use of capillary columns and new inlet technology using splitless or on-column sample introduction. The development of a sensitive, specific and reliable GLC method was essential for the ultimate completion of the proposed single dose pharmacokinetic study of drug-drug interactions with PF and also the *in vitro* and *in vivo* protein binding study of this drug.

1.13.2 Rationale for a Study of the Effect of Enzyme Induction by Cigarette Smoke and Phenobarbital

More than 200 drugs and chemicals are known to induce the microsomal drug-metabolizing enzymes in the liver [Conney, 1967; Hunter and Chasseaud, 1976; Greim, 1981]. These inducers have been classified according to their effects on various components of the enzyme system. The most common and simple categorization is classification of inducers into two groups. The first group, exemplified by phenobarbital, other barbiturates and rifampin, stimulates cytochrome P-450, the terminal oxidase of the microsomal mixed function oxidases, and induces a wide variety of metabolic pathways including oxidation, reduction and glucuronidation [Valerion *et al.*, 1974; Ioannides and Parke, 1975; Greim, 1981]. The second group, typified by benzo(a)pyrene, 3-methylcholanthrene and polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke, stimulates cytochrome P-448 (or P₁-450) and induces a more limited group of reactions [Beckett and Triggs, 1967; Conney, 1971; Jusko, 1978; Jusko, 1979; Vestal and Wood, 1980]. Finally, a third group, typified by pregnenolone 16 α -carbonitrile and other steroids, has been noted to also induce cytochrome P-450 [Solymoss *et al.*, 1971].

The enzymes involve in both phase I (biotransformation) and phase II (conjugation) reactions exist in multiple forms (*i.e.*, a family of

isozymes) [Lu and West, 1980]. The multiple forms of cytochrome P-450 in the liver microsomes of different species, such as rat, mouse, rabbit and human, have been identified and designated as P450I-P450XXII [Nebert *et al.*, 1987]. Enzyme inducers can increase the enzyme activity of a large number of routes of metabolism, or be relatively selective for individual isozymes [Breimer *et al.*, 1977]. Phenobarbital is one of the most potent barbiturates known to induce a large number of both microsomal and cytosolic enzymatic reactions. The metabolic pathways induced by phenobarbital include aromatic hydroxylation (e.g. 3,4-benz(a)pyrene), aliphatic hydroxylation (e.g. testosterone), O-dealkylation (e.g. acetophenetidine), N-dealkylation (e.g. aminopyrine), sulfoxidation (e.g. chlorpromazine), dehalogenation (e.g. halothane), nitro group reduction (e.g. chloramphenicol), azo link reduction (e.g. neoprontosil), de-esterification (e.g. procaine) and glucuronidation (e.g. salicylamide) [Conney *et al.*, 1967].

Branch and Herman [1984] reviewed the complex nature of events surrounding the effects of metabolic induction of the rapidly cleared β -adrenergic receptor blockers by phenobarbital and rifampicin. It had been well recognized that such changes in pharmacokinetic disposition might be very complex since inducing agents could influence liver size [Fouts and Rogers, 1965; Conney, 1967; Argyris, 1968], liver blood flow [Nies *et al.*, 1976], biliary flow [Klaassen, 1969; Klaassen, 1975] and protein binding [Bai and Abramson, 1982], in addition to their effect on drug metabolizing activity [Conney, 1967; Parke, 1975]. Furthermore, enzyme induction exhibited both dose and time dependency relationships [Breckenridge *et al.*, 1972; Mignet *et al.*, 1977; Ohnhaus *et al.*, 1977; Ohnhaus and Park, 1979; Ohnhaus *et al.*, 1983]. With such high clearance drugs it had been

recognized that the effect of enzyme induction was quite different when the drug was administered intravenously as compared to the oral route, due to substantial differences in the extent of first-pass metabolism after different routes of administration [Wilkinson and Shand, 1975].

Pretreatment with phenobarbital was shown to have much less effect on the kinetics of i.v. administered β -adrenergic blockers while a dramatic reduction in peak concentrations and AUC values for intact drug was observed after oral administration, although there was little observed effect on biological half-life [Wilkinson and Shand, 1975]. Drugs such as metoprolol [Bennett *et al.*, 1982], alprenolol [Collste *et al.*, 1979] and propranolol [Herman *et al.*, 1982; Herman *et al.*, 1983] exhibited increased oral clearance ranging from 50 to 500% after enzyme induction. Since PF showed a close structural resemblance to propranolol, shared modest β -blocking activity [Muller-Peltzer *et al.*, 1983; McLeod *et al.*, 1984] with the previously mentioned β -blockers and displayed extensive first-pass metabolism after oral dosing, it seemed likely that its oral clearance would be altered by phenobarbital treatment.

Tobacco smoke is a mixture of over 3000 chemicals [Kilburn, 1974; Severson *et al.*, 1976; Schumaker *et al.*, 1977]. Numerous compounds found in tobacco smoke, including nicotine [Wenzel and Broadie, 1966; Yamamoto *et al.*, 1966] and PAHs (e.g. benz(a)pyrene, anthracene, benz(a)anthracene, dibenz(a,h)anthracene, chrysene, 3,4-benzofluorene, fluoranthene and pyrene, produced from incomplete combustion of tobacco) [Welch *et al.*, 1969; Severson *et al.*, 1976], are potent enzyme inducing agents which increase microsomal enzyme activity. Unlike phenobarbital which affects different aspects of liver function and blood flow, the effect of PAHs seems to be limited to induction of selected drug metabolizing enzymes

[Conney, 1967; Klaassen, 1969; Klaassen, 1975; Parke, 1975; Nies *et al.*, 1976]. Numerous studies [Vestal *et al.*, 1975; Kapitulnik *et al.*, 1977; Kuntzman *et al.*, 1977; Cusack *et al.*, 1979; Vestal *et al.*, 1979; Wood *et al.*, 1979b; Grygiel *et al.*, 1981] and review articles [Jusko, 1978; Jusko, 1979; Vestal and Wood, 1980; Dawson and Vestal, 1982; D'Arcy, 1984] have reported or documented induced metabolic pathways of certain drugs in 'heavy' cigarette smokers. However, other drugs which are metabolized through the same pathways have been reported in two reviews to be unaffected by smoking [Jusko, 1978; Jusko, 1979]. For example, N-dealkylation has been found to be both induced (*e.g.* theophylline, imipramine) and unaffected (*e.g.* diazepam, meperidine, nortriptyline) by cigarette smoking. Similarly, aromatic hydroxylation has also be found to be induced (*e.g.* benzo(a)pyrene, zoxazolamine) and unaffected (*e.g.* phenytoin, warfarin) by cigarette smoke. This is possibly due to the presence of enzyme inhibitors (*e.g.* carbon monoxide, hydrogen cyanide) in cigarette smoke which may counteract the enzyme induction effect [Roth and Rubin, 1976].

The effect of cigarette smoke may increase the metabolism of PF in a manner similar to that observed for propranolol due to the structural and kinetic similarities between these two drugs. When the effect of smoking on propranolol kinetics was examined in groups of young and elderly healthy subjects [Vestal *et al.*, 1979], there was a three-fold larger oral clearance in the young smokers than that seen in the elderly smokers and non-smokers. These results suggested the possibility of a diminished inducibility of drug-metabolizing enzyme with aging.

In healthy volunteers, an oral dose of PF is virtually completely metabolized to a number of metabolites [Hege *et al.*, 1984a] by pathways

previously shown to be subject to the effect of phenobarbital and cigarette smoke with other drugs [Jusko, 1978]. No reports have yet appeared pertaining to the effects of enzyme induction on the pharmacokinetics of PF and its major and active metabolite, 5-hydroxy PF.

1.13.3 Rationale for a Study of the Serum Protein Binding of Propafenone

A drug in plasma can bind to protein(s) and it is widely accepted that only the free (unbound) drug can permeate cell membranes, reach receptor site(s) and exert pharmacological effect(s) [Goldstein, 1949]. Therefore, drug effect may be more closely related to free drug concentration than total (bound + unbound) drug concentration in blood. This is supported by the observations that free propranolol [McDevitt and Shand, 1975; McDevitt *et al.*, 1976] and disopyramide [Huang and Oie, 1981; Lima *et al.*, 1981] concentrations in plasma correlate better with the pharmacological effect than does total plasma drug concentration. Propafenone is highly plasma protein bound and there has been a suggestion that this binding may be concentration-dependent [Hollmann *et al.*, 1983b]. Moreover, PF appears to exhibit a steep dose-response effect [Connolly *et al.*, 1983b; Siddoway *et al.*, 1984a], although a good correlation between serum concentration and antiarrhythmic effect has yet to be established. If PF does exhibit concentration-dependent binding, it is possible that the large interindividual variability in the PF concentration necessary for arrhythmia suppression may be due, in part, to fluctuations in free PF serum concentration. The major active metabolite of PF, 5-hydroxy PF, may also contribute to the overall pharmacological effect [Connolly *et al.*, 1983b]. Steady-state PF concentrations may be affected by drug concentration-dependent binding or the concentration of plasma proteins.

Gillis *et al.* [1985] have shown that PF, like other basic drugs, binds to α_1 -acid glycoprotein (AAG). Alpha₁-acid glycoprotein is an acute-phase reactant plasma protein found in concentrations of 70-110 mg/dL in healthy individuals. It is characterized by a nonlinear, concentration-dependent and saturable binding which leads to a rapid increase in free fraction with increasing concentration of the drug in plasma beyond the 'saturation point'. Although its physiological function is unclear, serum AAG concentrations are greatly elevated in patients with Crohn's disease [Piafsky *et al.*, 1978], arthritis [Piafsky *et al.*, 1978], uremia [Henriksen *et al.*, 1982], traumatic injury [Edwards *et al.*, 1981] and following surgery [Aronsen *et al.*, 1972] or myocardial infarction [Johansson *et al.*, 1972; Snyder *et al.*, 1975]. Gillis *et al.* [1985] also demonstrated that PF binds to AAG to a greater extent than lidocaine, verapamil and propranolol, indicating that AAG may be an important binding protein for PF. In certain disease states when the concentration of AAG is increased [Keyser, 1979; Piafsky, 1980; Kremer *et al.*, 1988] such as renal failure, PF free fraction or free plasma concentration could be expected to decrease and a potential alteration in antiarrhythmic effect could result. Alteration in binding of PF due to altered AAG levels could also change the distribution and pharmacokinetic properties of this drug.

While an earlier report [Hollmann *et al.*, 1983b] has suggested concentration dependence of PF binding, the few selective drug concentrations studied have not establish whether or not PF undergoes non-linear binding over the apparent therapeutic range of drug concentration. Furthermore, no previous report has discussed the precise details of the binding, namely, the number of binding sites, the association constants and the binding capacity of the protein responsible

for the binding of this drug.

1.13.4 Rationale for a Study of the Pharmacological Effect of Propafenone

Many antiarrhythmic drugs including quinidine [Data *et al.*, 1976], lidocaine [Harrison *et al.*, 1971] and tocainide [Meffin *et al.*, 1977] are characterized by steep log dose-response relationships and evidence suggests that such is also the case for disopyramide [Niarchos, 1976; Robert *et al.*, 1978; Aitio, 1981]. Early studies have shown marked intersubject variability and an inconclusive relationship between PF concentration and effect [Keller *et al.*, 1978]. Most recently, Connolly *et al.* [1983b] have demonstrated that PF exhibits a similar degree of intersubject variability in concentration-response relationships to that seen for tocainide [Meffin *et al.*, 1977].

As mentioned in Section 1.13.3., theory suggests that free concentration of drug in plasma is more closely correlated with the pharmacological effect (therapeutic and/or toxic) than is total drug concentration. It is also mentioned in Section 1.13.3. that fluctuations in free PF serum concentration and/or the presence of the active metabolite (5-hydroxy PF) in serum may contribute to the wide interindividual variability in the PF concentration necessary for arrhythmia suppression. Since PF shows considerable variation in its pharmacokinetic parameters and pharmacological response, it is essential to establish the concentration-response relationship of PF in patients receiving this drug.

1.14 Objectives

- (a) to develop a sensitive and reproducible fused-silica capillary GLC-ECD assay method for the quantitation of PF and its major and active metabolite, 5-hydroxy PF, in small volumes of human biological fluids;
- (b) to identify the HFB derivative of PF and 5-hydroxy PF by GLC-MS;
- (c) to validate the developed GLC-ECD method by comparison with a published HPLC method;
- (d) to apply the developed GLC-ECD method to measure trough plasma PF concentrations in patients receiving PF for treatment of arrhythmia;
- (e) to study the concentration-dependency of the serum protein binding of PF *in vitro* using equilibrium dialysis;
- (f) to determine the plasma protein binding characteristics of PF, such as the association constant and the binding capacity of the protein;
- (g) to study the binding of PF in uremic serum as an example of plasma binding in disease;
- (h) to investigate the importance of AAG as a binding protein for PF and to study the correlation between serum AAG concentration and PF binding ratio;
- (i) to study PF pharmacokinetics and its metabolic induction by the prototype enzyme inducer, phenobarbital, in healthy non-smokers and heavy cigarette smokers;
- (j) to determine the effect of twenty-three days of phenobarbital treatment on serum AAG concentration and PF free fraction in healthy non-smokers and smokers;
- (k) to determine PF salivary concentration and its relationship with

PF serum total and free concentration and to evaluate the validity of salivary PF measurement; and

- (1) to evaluate the serum concentration-response relationship of PF - correlation between pharmacological effect and PF serum total concentration, 5-hydroxy PF serum total concentration or PF serum free concentration in patients receiving PF in the treatment of arrhythmia.

2. EXPERIMENTAL

2.1 Materials and Supplies

2.1.1 Drugs, Metabolites and Internal Standards

Propafenone hydrochloride, 5-hydroxy propafenone hydrochloride, 5-hydroxy-4-methoxy propafenone hydrochloride, Li-1115 hydrochloride, (internal standard, I.S.-a for propafenone quantitation) and Li-1548 hydrochloride (I.S.-b for 5-hydroxy propafenone quantitation) were supplied by Knoll Pharmaceuticals Canada Inc., Markham, Ontario, Canada.

Phenobarbital tablets (100 mg, Lot K5074HI) were obtained from the Family Practice Unit Pharmacy, U.B.C., Vancouver, B.C., Canada.

Sodium Chloride Injection USP (20 mL, 9 mg/mL) was purchased from Abbott Laboratories Ltd., Montreal, Canada and heparin sodium injection USP (10 mL, 1000 units/mL) from Allen & Hanburys, A Glaxo Canada Ltd. Co., Toronto, Ontario, Canada.

2.1.2 Chemicals and Reagents

Triethylamine (TEA) (SequanalTM grade), trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). ACS reagent grade sodium hydroxide was obtained from Fisher Scientific Co., Fair Lawn, NJ, U.S.A.; ACS reagent grade monopotassium phosphate, disodium phosphate, sodium carbonate and potassium carbonate from BDH Chemicals, Toronto, Ontario, Canada; ACS reagent grade hydrochloric acid from American Scientific and Chemical, Seattle, WA, U.S.A.; ACS reagent grade Ammonia Strong Solution from Mallinckrodt Inc.,

St. Louis, MI, U.S.A. and ACS reagent grade trichloroacetic acid from J.T. Baker Chemical Co., Phillipsburg, NJ, U.S.A. β -Glucuronidase/arylsulfatase (from *Helix pomatia*) was purchased from Boehringer Mannheim, West Germany.

2.1.3 Solvents

Benzene, toluene, methanol, dichloromethane and isopropyl alcohol (distilled in glass) and HPLC grade acetonitrile (ultraviolet (UV) cutoff 190 nm) were purchased from Caledon Laboratories Ltd., Georgetown, Ontario, Canada. Deionized distilled water was produced in the laboratory using a Milli-RO^R Water System (Millipore Corp., Bedford, MA., U.S.A.).

2.1.4 Gases

Ultra high purity (UHP) hydrogen and argon/methane (95:5) were purchased from Matheson Gas Products Canada Ltd., Edmonton, Alberta, Canada and nitrogen, U.S.P., from Union Carbide Canada Ltd., Toronto, Ontario, Canada.

2.1.5 Radial Immunodiffusion Plates

NOR-Partigen^R AAG radial immunodiffusion (RID) plates (12-well, volume = 5 μ L per well) containing monospecific antiserum to human AAG in a ready-for use agarose-gel layer were purchased from Terochem Laboratories Ltd., Edmonton, Alberta, Canada.

2.1.6 Ultrafiltration Device

Disposable ultrafiltration devices (CentrifreeTM Micropartition System) were purchased from Amicon Canada Ltd., Oakville, Ontario, Canada. Each ultrafiltration device consisted of a single YMT membrane (molecular

weight (M.W.) cutoff = 30,000 Daltons) and an O-ring, sealed between the sample reservoir and support base. The reservoir was provided with a cap to minimize sample evaporation and pH change due to loss of carbon dioxide. A removable filtrate collection cup was attached to the support base.

2.1.7 Equilibrium Dialysis Device

Plexi-Glass^R dialysis cells (0.4 mL and 1.0 mL) were used for equilibrium dialysis. Cellophane dialysis membrane "sacks" (M.W. cutoff = 12,000 Daltons) were purchased from Sigma Chemical Co., St. Louis, U.S.A.

2.1.8 Other Supplies

Pyrex^R disposable glass culture tubes (15 mL) were purchased from Corning Glass Works, Corning, NY, U.S.A. and polytetrafluoroethylene (PTFE) lined screw caps from Canlab, Vancouver, B.C., Canada.

VenisystemsTM Butterfly^R-19 INT cannulae were purchased from Abbott Laboratories, Ltd., Montreal, Canada. Vacutainer^R blood collection tubes (without additive) were obtained from Becton Dickinson Canada Inc., Mississauga, Ontario, Canada.

2.2 Columns

2.2.1 GLC Column

A bonded-phase fused-silica capillary column, 25 m x 0.31 mm I.D., was used for all GLC and GLC-MS analyses (stationary phase, cross-linked 5% phenylmethyl-silicone, film thickness 0.52 μm ; phase ratio 150; Hewlett-Packard, Palo Alto, CA, U.S.A.).

2.2.2 HPLC Column

An UltrasphereTM-cyano column, 15 cm x 4.6 mm I.D. (5 μ dp), Beckman Instruments, Inc., Altex Div., San Ramon, CA, U.S.A., was used for HPLC analyses.

2.3 Equipment

2.3.1 Gas-Liquid Chromatography

GLC analyses were performed on a Model 5830A Hewlett-Packard (HP) gas-liquid chromatograph, equipped with a Model 18835B capillary inlet system, a ⁶³Ni electron-capture detector, a Model 18850A GC terminal for peak integration and a Model 7671A automatic sampler. A splitless injection mode was used, employing a fused-silica inlet liner with a small plug of silanized glass-wool 3 cm from the column end. ThermogreenTM LB-2 septa (Supelco, Inc., Bellefonte, PA, U.S.A.), low-bleed septa at high inlet temperatures, were used. The septum was changed routinely to prevent leakage resulting from repeated puncturing during automatic sampling.

2.3.2 Gas Liquid Chromatography-Mass Spectrometry

GLC-MS analyses were obtained using an HP Model 5987A gas-liquid chromatography-MS system. The data were processed by a Series 1000E HP computer and displayed on an HP Model 2623A terminal. Identical capillary columns were used for GLC-MS analyses, as in GLC analyses.

2.3.3 High-Performance Liquid Chromatography

A HP Model 1090 liquid chromatograph equipped with a HP Model 1040A

diode-array UV detector and a Model 310 HP computer for data analysis was used.

2.3.4 Miscellaneous

Other equipment used included: Eppendorf micropipettes, a Vortex-Genie^R mixer (Fisher Scientific Industries, Springfield, MA, U.S.A.), a pH meter and electrode (Fisher Scientific Co., Springfield, MA, U.S.A.), a Model 415-110 Labquake^R rotary shaker (Labindustries, Berkley, CA, U.S.A.), an incubation oven (Isotemp^R model 350, Fisher Scientific Industries, Springfield, MA, U.S.A.), an IEC model 2K centrifuge (Damon/IEC Division, Needham Hts., MA., U.S.A.) and a Behringwerke measuring viewer (Behringwerke AG, Marburg, West Germany).

2.4 Preparation of Stock and Reagent Solutions

2.4.1 Drug, Metabolites and Internal Standard

Propafenone hydrochloride was accurately weighed and dissolved in deionized distilled water using serial dilution to a final concentration of ~100 ng/mL (~11.07 mg of PF hydrochloride is equivalent to ~10 mg of PF free base).

5-Hydroxy propafenone hydrochloride was accurately weighed and dissolved in methanol:deionized distilled water (1:9) using serial dilution to a final concentration of ~100 ng/mL (~11.02 mg of 5-hydroxy PF hydrochloride is equivalent to ~10 mg of 5-hydroxy PF free base).

5-Hydroxy-4-methoxy propafenone hydrochloride was accurately weighed and dissolved in methanol:deionized distilled water (1:9) using serial

dilution to a final concentration of ~100 ng/mL (~10.94 mg of 5-hydroxy-4-methoxy PF hydrochloride is equivalent to ~10 mg of 5-hydroxy-4-methoxy PF free base).

Li-1115 hydrochloride was accurately weighed and dissolved in deionized distilled water using serial dilution to a final concentration of ~200 ng/mL (~11.11 mg of Li-1115 hydrochloride is equivalent to ~10 mg of Li-1115 free base).

Li-1548 hydrochloride was accurately weighed and dissolved in deionized distilled water using serial dilution to a final concentration of ~200 ng/mL (~11.06 mg of Li-1548 hydrochloride is equivalent to ~10 mg of Li-1548 free base).

All stock and diluted solutions were protected from sunlight by wrapping the glass containers in aluminum foil and stored at 4°C after preparation, for up to four months.

2.4.2 Reagents and Solutions

Triethylamine 0.003 M was prepared by diluting triethylamine with toluene. Four or five pellets of NaOH were added to the solution.

Sodium hydroxide (NaOH) 1 M and 5 M solutions were prepared by dissolving NaOH pellets in deionized distilled water.

Sodium carbonate (Na_2CO_3) 0.1 M solution and potassium carbonate (K_2CO_3) 5 M solution were prepared by dissolving Na_2CO_3 and K_2CO_3 powder in deionized distilled water.

Hydrochloric acid (HCl) 1 M was prepared by diluting ACS reagent grade concentrated (37%) HCl in deionized distilled water.

Ammonium hydroxide (NH_4OH) 4% was prepared by diluting Ammonia Solution Strong (27%) in deionized distilled water.

Phosphate buffer (pH 7.4) was prepared using the following procedures. Monopotassium phosphate (KH_2PO_4 , 2.28 g) was accurately weighed and dissolved in deionized distilled water to a final volume of 250 mL (solution A). Disodium phosphate (Na_2HPO_4 , 9.47 g) was accurately weighed and dissolved in deionized distilled water to a final volume of 1 L (solution B). An accurate volume of solution A (200 mL) was transferred into a clean 1-litre volumetric flask. The volume was then adjusted to 1 L with stock solution B to yield a phosphate buffer of 0.067 M. The pH of the final solution was checked and adjusted, if necessary, to 7.4 with aliquots of stock solutions A or B.

Phosphate buffer (pH 6.0) was prepared by dissolving 2.28 g of KH_2PO_4 in deionized distilled water to a volume of 250 mL (solution 1) and 0.95 g Na_2HPO_4 to a volume of 100 mL (solution 2). The final buffer solution was prepared by combining solutions 1 and 2 (90 mL of solution 1 and 10 mL of solution 2).

2.5 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of Propafenone

2.5.1 Preliminary Development of a Capillary GLC-ECD Assay Method for Propafenone

2.5.1.1 Extraction and Trifluoroacetic Anhydride Derivatization Treatment for Propafenone

An aliquot of blank serum spiked with PF and I.S.-a in desired concentrations was made to a constant volume with water. The solution was

alkalinized with 0.5 mL of 1 M sodium hydroxide to pH 11 and extracted with 6 mL of benzene. After rotary mixing for 20 min and centrifugation, the benzene layer was transferred to a clean glass tube, to which 2 mL of 1 M hydrochloric acid was added. The solution was then rotary mixed for 20 min and after centrifugation, the benzene layer was discarded and the aqueous layer was washed with two 4 mL aliquots of benzene. Then 0.5 mL of 5 M sodium hydroxide and 6 mL of benzene were added to the aqueous layer and the solution rotary mixed for 20 min. After centrifugation, the benzene layer was transferred to a clean test tube and dried under nitrogen in a 40°C water bath to complete dryness. The dried sample was reconstituted with 800 μ L of toluene (0.003 M TEA was added as a catalyst for the derivatization reaction) and derivatized with TFAA at 65°C for 1 h.

2.5.1.2 Neutralization of Excess Trifluoroacetic Anhydride

Hydrolysis of the excess TFAA to TFA-acid was carried out by mixing the sample with 0.5 mL of water on a vortex mixer for 10 sec, followed by neutralization of the acid with 0.5 mL of 4% ammonium hydroxide for 15 sec.

2.5.2 Optimal Derivatization Conditions For Propafenone

2.5.2.1 Use of Triethylamine as Catalyst and Optimal Derivatization Time

Two sets of samples containing equivalent amounts of PF and I.S.-a in methanol were dried and reconstituted with 800 μ L of toluene, with TEA (0.003 M) added to only one set. The samples were then derivatized with 50 μ L of HFBA for various times (0.5, 1, 2, 3 and 4 h) at 65°C.

The peak areas of the PF derivative were evaluated to assess the necessity of using TEA as a catalyst and to determine the optimum

derivatization time.

2.5.2.2 Quantity of Triethylamine Used

Samples containing equivalent amounts of PF and I.S.-a in methanol were dried and reconstituted with various volumes (200, 400, 600 and 800 μL) of toluene containing 0.003 M TEA. The samples were then derivatized with 50 μL of HFBA at 65°C for 1 h.

The peak areas of the HFB-PF derivative were evaluated to determine the minimum amount of TEA required as a catalyst for the derivatization reaction.

2.5.2.3 Quantity of Heptafluorobutyric Anhydride Used

Samples containing equivalent amounts of PF and I.S.-a in methanol were dried and reconstituted with 800 μL of toluene containing 0.003 M TEA. The samples were then derivatized with various volumes (5, 10, 20 and 40 μL) of HFBA at 65°C for 1 h.

The peak areas of the HFB-PF derivative were evaluated to determine the minimum amount of HFBA required for the derivatization reaction.

2.5.3 Solvent Extraction Efficiency

The optimal extraction solvent was tested by adding identical concentrations of PF to blank plasma and subsequent extraction with four different solvents, *viz.*, toluene, benzene, hexane or a combination of toluene:dichloromethane:isopropyl alcohol (7:3:1) [Brode *et al.*, 1984]. After extraction, an identical concentration of I.S.-a in methanol was added to the above extracted samples and also to a sample that contained the same concentration of PF in methanol (not subjected to extraction).

The samples were dried under nitrogen and then derivatized with HFBA. The extraction efficiency of these solvents was evaluated by comparing the peak area ratios of the extracted samples to the peak area ratio of the unextracted sample.

2.5.4 Optimal GLC-ECD Conditions

The optimal column temperature was determined by injecting a sample containing HFB derivatives of PF and I.S.-a into the GLC under different temperature programming conditions to obtain optimum peak shape and peak symmetry for both PF and I.S.-a as well as resolution of the peaks for PF and I.S.-a from peaks of serum endogenous substances.

Various inlet purge activation times (10, 20, 40, 60 and 80 sec), injection port temperatures (200, 210, 220, 230, 240, 250 and 260°C), detector temperatures (330, 340 and 350°C) and make-up gas flow rates (30, 40, 50 and 60 mL/min) were examined as mentioned previously. The peak areas of HFB-PF or peak area ratio (HFB-PF/HFB-I.S.-a), peak shape and symmetry were examined to obtain the optimal value for each parameter.

2.5.5 Recovery of Propafenone

2.5.5.1 Extractability of Propafenone

Propafenone hydrochloride was dissolved in water and methanol, respectively, to produce solutions of equal concentrations (equivalent to 100 ng/mL of base). Various volumes (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL) of these solutions were transferred into two sets of glass tubes, respectively. To the set of PF hydrochloride samples in water was added human blank serum followed by extraction with benzene. The salt of the

I.S.-a was dissolved in methanol to yield a concentration of 200 ng/mL, and 0.35 mL of this solution was added to both sets of samples. The two sets of samples were then subjected to the same derivatization reaction. The amounts of PF extracted from the aqueous solutions by benzene were calculated from the calibration curve of PF hydrochloride in methanol and compared with the actual amount of PF added.

2.5.5.2 Variability in Recovery of Propafenone

Propafenone hydrochloride (equivalent to 0.1 mg/mL of base) in distilled water was diluted to prepare two solutions with concentrations of 10 and 100 ng/mL. Four parts, three parts, two parts and one part of the 10 ng/mL solution were mixed with one part, two parts, three parts and four parts of the 100 ng/mL solution, respectively, to yield solutions with concentrations of 28, 46, 64 and 82 ng/mL. One milliliter of each of these solutions, including the 10 and 100 ng/mL solutions were then subjected to analysis. The amount of PF in each solution was estimated from a calibration curve prepared at the same time. The amount of PF found was plotted versus the amount of PF added and the correlation was assessed by linear regression.

2.5.6 Quantitative Analysis of Propafenone

An aliquot of human biological fluid (serum or saliva) spiked with PF standard solutions and I.S.-a was extracted and derivatized. A 2 μ L aliquot of the final solution containing the HFBA derivatives of PF and I.S.-a was injected into the GLC under optimized conditions. A calibration curve was constructed by plotting the peak area ratio (HFB-PF/HFB-I.S.-a) versus the amount of PF added, using linear regression. Different

concentration ranges were used for various samples, viz., 0.5-10 ng/mL for single dose volunteer saliva samples, 2.5-50 ng/mL for single dose volunteer serum samples and 10-100 ng/mL for steady-state patient serum samples. The calibration curve thus obtained was used for the estimation of the unknown concentrations of PF in biological samples (an equivalent amount of I.S.-a was added to both the standard solutions and the unknown samples). All standard solutions and unknown samples were analyzed in duplicate and each sample was injected twice into the GLC.

2.5.7 Determination of Day-to-Day Variability

The day-to-day variability of the GLC-ECD method was determined by spiking blank plasma with an equivalent amount of PF which was then stored at -20°C. On the day of analysis, a selection of these samples was analyzed along with the standard solutions and unknown plasma samples by spiking them with an equivalent amount of I.S.-a. The coefficients of variation were calculated by comparing the amount of PF found to the amount of PF added and the within-run and between-run precision were determined.

2.6 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of 5-Hydroxy Propafenone and 5-Hydroxy-4-Methoxy Propafenone

2.6.1 Extraction, Heptafluorobutyric Anhydride Treatment and Neutralization of Excess Heptafluorobutyric Anhydride

The GLC-ECD method developed for the analysis of PF was modified for the analysis of 5-hydroxy PF and 5-hydroxy-4-methoxy PF. An aliquot of blank serum spiked with 5-hydroxy PF or 5-hydroxy-4-methoxy PF and I.S.-b

in desired concentrations was diluted to a constant volume with water. The solution was alkalized with 0.15 mL of 0.1 M sodium carbonate to pH 10 and extracted with 6 mL of a solvent mixture, toluene:dichloromethane:isopropyl alcohol (7:3:1). After rotary mixing for 20 min and centrifugation, the organic layer was transferred to a clean glass tube, and 2 mL of 1 M hydrochloric acid were added. The solutions were then rotary mixed for 20 min and after centrifugation, the organic layer was discarded, and the aqueous layer washed with two 4 mL aliquots of the solvent mixture. Then 0.5 mL of 5 M potassium carbonate and 6 mL of the solvent mixture were added to the aqueous layer and the solution rotary mixed for 20 min. After centrifugation, the organic layer was transferred to a clean glass tube and dried completely under nitrogen at 40°C in a water bath. The dried sample was reconstituted with 800 μ L of toluene (0.003 M TEA was added as a catalyst for the derivatization reaction) and derivatized with HFBA at 65°C for 1 h. Excess HFBA was removed by mixing the sample with 0.5 mL phosphate buffer (pH 6) for 30 sec. The toluene layer was transferred to a clean autosampler vial, diluted with toluene and capped with a PTFE-lined seal. A volume of 2 μ L of this mixture was then injected into the GLC for GLC-ECD analysis.

2.6.2 Optimal Derivatization Conditions for 5-Hydroxy Propafenone

2.6.2.1 Quantity of Heptafluorobutyric Anhydride Used

Samples containing equivalent amounts of 5-hydroxy PF and I.S.-b in methanol were dried under nitrogen and then reconstituted with 800 μ L of toluene containing 0.003 M TEA. The samples were then derivatized with various volumes (25, 50 and 100 μ L) of HFBA at 65°C for 1 h.

The peak areas of the HFB-5-hydroxy PF derivative were evaluated to determine the minimum quantity of HFBA required for the reaction.

2.6.2.2 Derivatization Time

Two sets of samples containing equivalent amounts of 5-hydroxy PF and I.S.-b in methanol were dried under nitrogen and subsequently reconstituted with 400 μ L of toluene, with TEA (0.003 M) added to only one set. The samples were then derivatized with 20 μ L of HFBA for various times (5, 15, 30, 45 and 60 min) at 65°C.

The peak areas of the HFB-5-hydroxy PF derivative were evaluated to assess the necessity of using TEA as a catalyst and to determine the optimum derivatization time.

2.6.3 Extraction Efficiency of Solvents

The optimal extraction solvent was tested by adding identical concentrations of 5-hydroxy PF to blank plasma and subsequent extraction with four different solvents, *viz.*, toluene, benzene, hexane and a combination of toluene:dichloromethane:isopropyl alcohol (7:3:1). After extraction, an identical concentration of I.S.-b in methanol was added to the extracted samples and also to a sample that contained the same concentration of 5-hydroxy PF in methanol (not subjected to extraction). The samples were dried under nitrogen and then derivatized. The extraction efficiency of these solvents was evaluated by comparing the peak area ratios of the extracted samples to the peak area ratio of the unextracted sample.

2.6.4 Optimal GLC-ECD Conditions for 5-Hydroxy Propafenone

The optimal column temperature was determined by injecting a sample containing the HFB derivatives of PF, 5-hydroxy PF and I.S.-b into the GLC under different temperature programming conditions to obtain ideal peak symmetry and resolution for 5-hydroxy PF and I.S.-b, as well as complete separation of 5-hydroxy PF and I.S.-b from each other and from PF and endogenous substances present in biological fluids.

2.6.5 Recovery of 5-Hydroxy Propafenone

The hydrochloride salt of 5-hydroxy PF was dissolved in water and methanol, respectively, to make up solutions of equal concentrations (equivalent to 100 ng/mL of base). Various volumes (0.1, 0.2, 0.3, 0.4 and 0.5 mL) of these solutions were transferred into two sets of glass tubes, respectively. Human blank serum was added to the set of 5-hydroxy PF hydrochloride samples in water, followed by extraction with toluene: dichloromethane:isopropyl alcohol (7:3:1). The salt of I.S.-b was dissolved in methanol to yield a concentration of 200 ng/mL, and 0.2 mL of this solution was added to both sets of samples. The two sets of samples were then subjected to the same derivatization reaction. The amount of 5-hydroxy PF extracted from the aqueous solutions by the solvent mixture were calculated from the calibration curve of 5-hydroxy PF hydrochloride in methanol and compared with the actual amount of 5-hydroxy PF added.

2.7 Structural Confirmation of the HFB Derivatives of Propafenone, 5-Hydroxy Propafenone and I.S.-a by Gas-Liquid Chromatography-Mass Spectrometric Analysis

Structural confirmation of the derivatives, HFB-PF and HFB-I.S.-a, was carried out by GLC-MS with electron-impact (EI), positive-ion chemical-ionization (PICI) and negative-ion chemical-ionization (NICI) as the GLC-MS ionization sources. HFB-5-hydroxy PF was analyzed by EI-GLC-MS. The GLC-MS operating conditions were the same as used in routine GLC analyses. Methane was used as reagent gas in PICI and NICI and the temperature of the GLC-MS interface was 270°C. The MS operating conditions were: electron ionization energy 70, 110 and 130 eV for EI, PICI and NICI-MS, respectively; emission current, 0.3 mA; ion source temperature, 240°C.

2.8 Measurement of Trough Plasma Propafenone Concentrations by Electron-Capture Detection Gas-Liquid Chromatography and High-Performance Liquid Chromatography in Patients Receiving Propafenone

Patient samples were obtained during the course of a study examining the effectiveness of PF in the treatment of atrial fibrillation and supraventricular tachycardia. Trough plasma samples were drawn on a treatment day immediately prior to the next daily dose. All samples were assayed in duplicate by the present GLC-ECD method. Propafenone concentrations of ten patient samples were also measured by a published HPLC method to test the validity of the GLC-ECD measurement technique. The samples selected were based on the sensitivity of the HPLC method and had a

PF concentration greater than 300 ng/mL. A minor modification of the method of Harapat and Kates [1982] was employed. The mobile phase used in the method of Harapat and Kates [acetonitrile:0.005 M phosphate buffer, pH 2.4, (25:75)] was changed slightly [acetonitrile:0.005 M phosphate buffer, pH 2.9, (38:62)] to reduce analysis time and to improve chromatographic peak shape.

2.9 *In Vitro* Serum Protein Binding Study

2.9.1 Equilibrium Time for Propafenone During Equilibrium Dialysis

Phosphate buffer (pH 7.4) containing PF was dialyzed against an equal volume (1 mL) of blank serum at 37°C for 1, 2, 4 and 6 h. The concentration of PF in the buffer was measured and calculated as percentage of the initial PF concentration and plotted against time. Equilibration is established when a plot of percent of initial concentration *versus* time declines to an apparent asymptote (*i.e.*, equilibration of free PF concentration between the serum and buffer chambers of the dialysis cell). Equilibrium time for PF was evaluated at both low (2.5 µg/mL) and high (100 µg/mL) PF concentrations.

2.9.2 Non-Specific Binding of Propafenone

The extent of adsorption of PF to the equilibrium dialysis membrane was determined by measuring PF concentration before and after soaking the membrane in buffer containing PF. The extent of adsorption of PF to the equilibrium dialysis cell was also determined by injecting buffer containing PF into cells lacking the membrane and by measuring PF

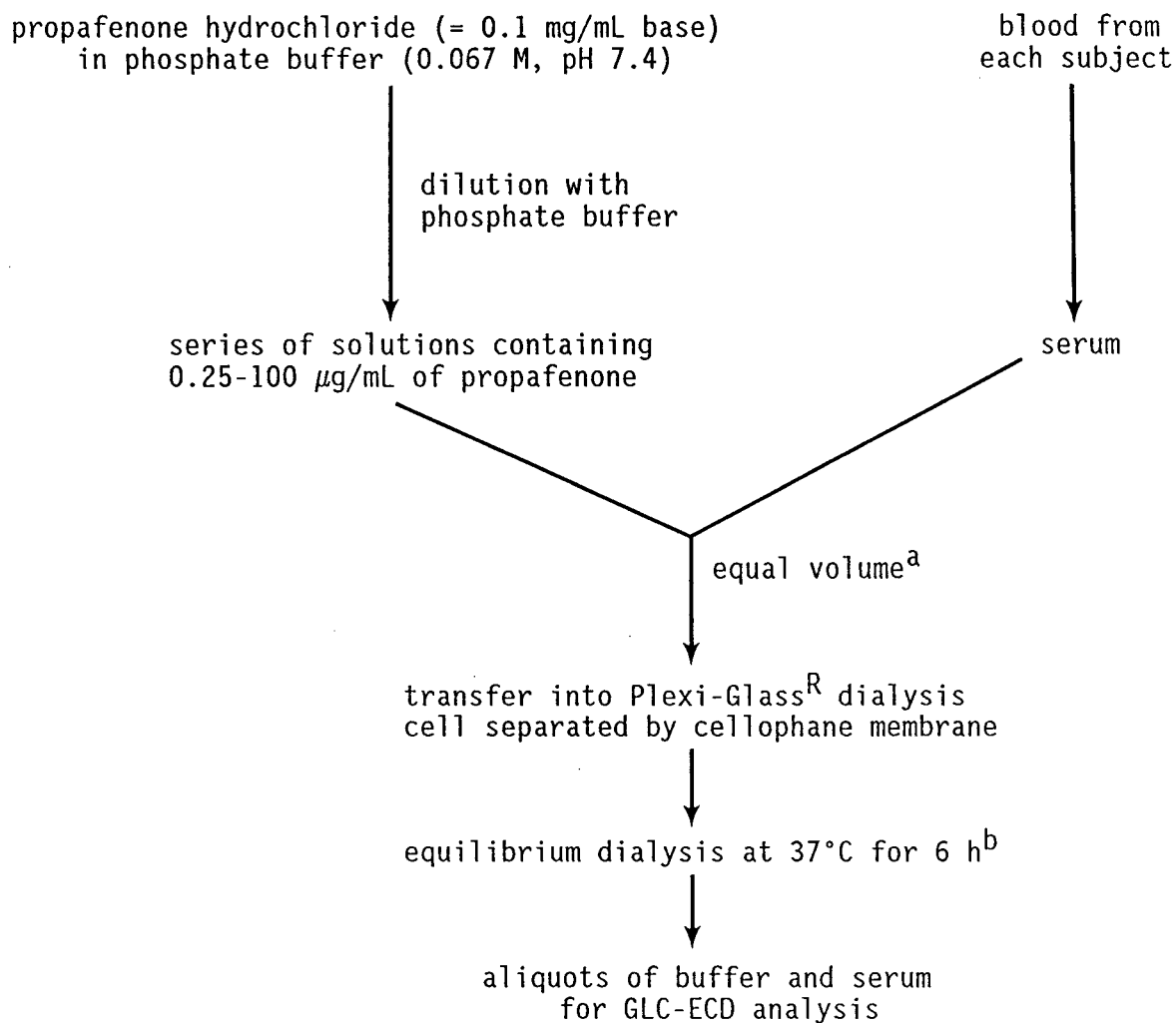
concentration in buffer before and after incubation in the cell for 6 h in a 37°C water bath.

The non-specific binding of PF to the ultrafiltration device was determined by measuring PF concentration before and after ultrafiltration (*i.e.*, PF concentration in ultrafiltrate). Ultrafiltration was carried out at 25°C with a centrifugal force of 2000 x g for 30 min. About 300-400 µL of ultrafiltrate were obtained after filtration of 1 mL of serum spiked with a small volume (5 µL) of buffer containing PF (0.1, 0.5, 1, 5, 10 and 100 µg/mL) to minimize dilution of protein concentration in the serum.

All non-specific binding studies were conducted at PF concentrations of 0.1, 0.5, 1, 5, 10 and 100 µg/mL and the percentage of PF adsorbed to material surfaces was calculated.

2.9.3 Equilibrium Dialysis Procedure

The equilibrium dialysis procedure is shown in Figure 3. The cellophane dialysis membrane was immersed in boiling distilled water for 1 h and then soaked in phosphate buffer (pH 7.4) for another hour before mounting within the dialysis cells. Extreme care was taken not to touch the surface of the membranes prior to, during and after the mounting procedure. Blank serum was dialyzed against an equal volume (0.4 mL for the concentration range of 1 to 100 µg/mL and 1 mL for the concentration range of 0.25 to 0.75 µg/mL) of phosphate buffer containing PF in a 37°C water bath with the assembly rotating at a speed of 14 rpm for 6 h. At the end of dialysis, aliquots of the buffer and serum were transferred to clean glass test tubes to which equivalent amounts of I.S.-a were added and the samples analyzed for PF concentration by GLC-ECD. Dialysis was performed in triplicate at each concentration. A drop of the dialysis buffer was



a 0.4 mL for PF concentration range of 1-100 µg/mL and

1.0 mL for PF concentration range of 0.25-0.75 µg/mL

b dialysis was performed in triplicate at each concentration in all
subjects

Figure 3. Scheme of equilibrium dialysis for propafenone

added to 0.1 mL of 3% trichloroacetic acid to test for leakage of protein across the membrane. The absence of turbidity was used as an indicator of membrane integrity. The free fraction of PF was calculated from the ratio of equilibrium PF concentration in the buffer and serum.

2.9.4 Determination of Propafenone Binding Parameters - Rosenthal Analysis

Venous blood was obtained from 6 non-smoking, healthy male Caucasians, aged 22-39 y, weight, 61-80 kg, height, 170-183 cm, using glass Vacutainers^R (containing no heparin or other anticoagulants) and an indwelling Butterfly^R-19 INT cannula inserted in the brachial vein. Precaution was taken so that collected blood did not come into contact with the red rubber cap of the Vacutainers^R. Following coagulation and centrifugation of the blood samples, the serum was separated and transferred to a glass tube with a PTFE lined screw cap and stored at -20°C. Equilibrium dialysis was performed using the collected serum within 2 weeks after blood collection. The free fraction of PF was determined over a concentration range from 0.25 to 100 µg/mL and was calculated by dividing the PF concentration in buffer (free drug concentration) by the PF concentration in serum (total drug concentration). The binding ratio (bound concentration/free concentration) of PF was plotted *versus* the bound concentration, by the method of Rosenthal [1967]. The computer program NONLIN [Metzler, 1974] was used to obtain the binding parameters of PF, namely the number of binding sites, the association constants and the binding capacity of the protein. 'Regression with replication' was carried out to test the linearity of free fraction over the PF concentration range studied; the level of significance of 0.05 was used.

2.9.5 Scatchard Plot of the Binding Data

The binding data from the six healthy subjects were also plotted by the method of Scatchard, with r' plotted *versus* $[r'/\text{free drug concentration}]$. The parameter r' is defined as the moles of drug bound divided by the moles of protein (*e.g.*, albumin, AAG, lipoprotein, etc.).

2.9.6 Propafenone Free Fraction in Pooled Uremic Serum

In preparation for the protein binding study, aliquots of sera obtained prior to hemodialysis from nine uremic dialysis patients (creatinine clearance, $CL_{Cr} < 10$ mL/min) were subjected to GLC analysis and shown to be free of substances capable of interfering with the measurement of PF. These sera were then pooled to yield a larger volume for an equilibrium dialysis study of the protein binding of PF in uremia. The free fraction of PF was determined over an initial total PF concentration range of 1 to 5 $\mu\text{g/mL}$.

2.9.7 Effect of Uremia and Renal Failure on the Serum Binding of Propafenone

The sera of three mid-range uremic patients ($CL_{Cr} = 36 \pm 12$ mL/min) and sera of five patients with chronic renal failure ($CL_{Cr} = 0$ mL/min) obtained from another PF study were used. The free fraction of PF was determined by equilibrium dialysis at an initial PF concentration of 1 $\mu\text{g/mL}$.

2.9.8 Measurement of Serum Protein Concentrations

2.9.8.1 Serum Albumin Concentration

Serum samples from the six healthy subjects were analyzed for serum albumin concentration by the Division of Clinical Chemistry, Department of Pathology, University Hospital, U.B.C. Site, Vancouver, B.C. Albumin concentration was assayed by a colorimetric test. The measurement was performed at 630 nm using a Kodak 700 Ektachem Control Unit, Eastman Kodak Company, Rochester, NY, U.S.A. The coefficient of variation was 2-3% (within an albumin concentration range of 2.5-4.8 g/dL).

2.9.8.2 Serum α_1 -Acid Glycoprotein Concentration

All samples were assayed for AAG concentration in our laboratory using a RID procedure with NOR-Partigen^R AAG-RID plates. The method was based on the principle that the precipitation ring formed due to the formation of the AAG-antisera complex was in a linear relationship with the AAG (antigen) concentration. Fresh undiluted serum samples (5 μ L) were transferred into the wells of the RID plates using an Eppendorf micropipette. Each sample was analyzed in triplicate. The plates were allowed to stand tightly closed at room temperature for 2 days to attain the diffusion end-point. The diameters of the precipitate rings were measured using a Behringwerke Measuring Viewer and the AAG concentrations read from a calibration table provided with the RID plates. The accuracy of the method, estimated by using the control serum for Nor-Partigen^R, was \pm 5% of the standard value provided by the company.

2.10 Phenobarbital Treatment in Non-Smokers and Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

2.10.1 Study Subjects

Eight healthy non-smoking (age 21-38 y, weight 65-82 kg, height 168-184 cm) and eight healthy smoking (age 25-43 y, weight 64-80 kg, height 173-185 cm) Caucasian males served as study subjects after giving written informed consent. The recruitment of these subjects was made through Campus bulletins and advertisement in local newspapers. The protocol and the procedures of the present study were approved by the University Human Ethics Committee, U.B.C. Each volunteer received Ethics Committee approved compensation (\$210) for inconvenience incurred during participation in the present research project.

None of the non-smoking subjects had a recent history of smoking (in the past 15 years). All smoking individuals were habitual cigarette smokers (none admitted marijuana use) who smoked at least 20 cigarettes (tar content: 8-16 mg, nicotine content: 0.8-1.1 mg) a day for the past 5 years. All subjects admitted to mild to moderate alcohol consumption. None had a history of cardiac, hepatic or renal diseases and all had normal physical examination, normal electrocardiogram (ECG) and biochemical/haematological laboratory results at the time of the study (Table 3). All subjects were instructed to abstain from other medications for 2 weeks prior to and during the study. Also, they were prohibited from consumption of alcohol or any caffeine containing beverages for 48 h before and during each phase of the study.

Table 3. Characteristics of non-smoking and smoking subjects*.

Subject	Age (y)	Weight (kg)	ALP ^a (IU/L)	SGOT ^b (IU/L)	CL _{cr} ^c (mL/min/1.73m ²)
Non-smokers					
BK	38	65.4	92	27	82.9
NP	28	70.4	55	25	122.6
CA	21	81.8	80	21	87.9
DA	21	72.7	105	46	92.3
SG	33	75.0	57	32	149.6
MV	24	72.7	85	47	119.9
GP	22	79.5	81	24	152.0
UH	34	65.0	87	27	63.6

Mean	28	72.8	80	31	108.9
±s.d.	±7	±6.0	±17	±10	±32.2
Smokers					
JL	43	77.2	66	18	128.5
MA	35	80.0	70	27	141.6
TN	33	63.5	35	20	149.4
DW	25	79.0	58	24	73.8
GE	26	75.0	67	24	95.0
MG	38	77.0	78	34	140.0
SR	35	75.0	96	19	173.4
DB	37	72.7	78	24	150.0

Mean	34	74.9	69	24	131.5
±s.d.	±6	±5.2	±18	±5	±32.2

a serum alkaline phosphatase, normal range = 30-110 IU/L

b serum glutamic oxaloacetic transaminase, normal range = 5-47 IU/L

c creatinine clearance, corrected for body surface area, normal range = 63-173 mL/min/1.73 m²

s.d. standard deviation

* smoked an average of 20 cigarettes (tar content: 8-16 mg; nicotine content: 0.8-1.1 mg) per day for the past 5 years

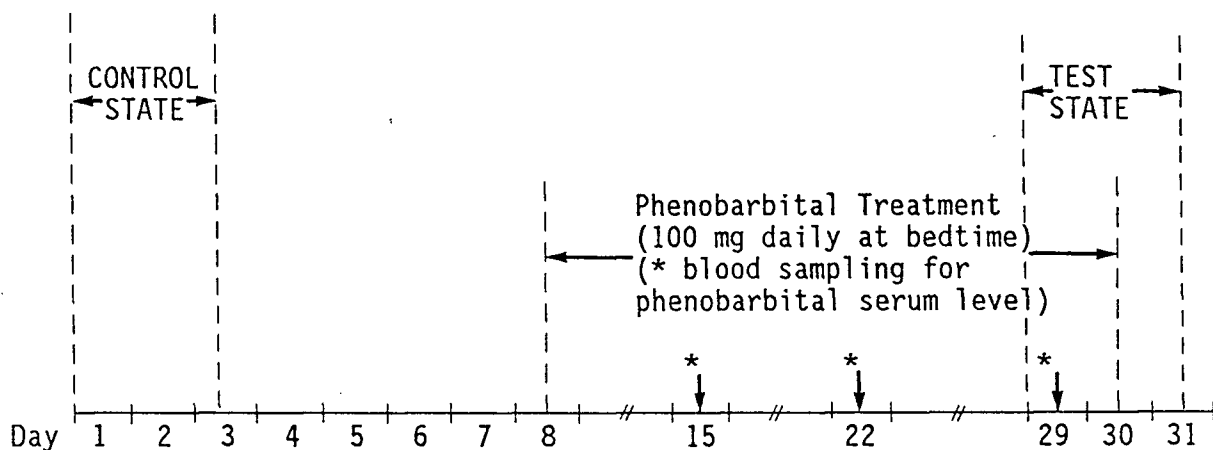
2.10.2 Study Protocol

The study protocol is shown in Figure 4. In the first phase of the study (control state), subjects received a 300 mg PF hydrochloride tablet orally. Blood, saliva and urine samples were collected up to 48 h after PF administration. After a 5 day washout period, subjects received phenobarbital (100 mg orally), at bedtime from day 8 to day 30. The second phase of the study (test state) was carried out on day 29. Again, each subject received a single oral dose of PF (300 mg). Biological fluid sampling and other study conditions were conducted in the same manner as employed in the control state.

2.10.3 Dosing, Biological Fluid Sampling and Physiological Monitoring of Study Subjects

During the two phases of the study (control state and test state), all subjects were under the supervision of a cardiologist in the Division of Cardiology, University Hospital, U.B.C. Site. All subjects fasted for the 12 h preceding the study. A butterfly cannula was inserted into the brachial vein of each subject on the day of the study for multiple blood sampling. Each subject received one tablet of PF hydrochloride (300 mg) orally with 200 mL of water and, thereafter, did not ingest food for 4 h or water for 3 h after PF administration. To minimize alterations in hepatic blood flow caused by postural changes, all subjects were required to remain seated on a chair for 60 min prior to and a minimum of 90 min after dosing. Blood pressure was measured and an ECG was recorded prior to and 1 h following PF administration. Venous blood (10 mL) and saliva (2-4 mL) samples were collected prior to the dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 30 and 48 h after PF administration. Serial urine

Day 1 to Day 31



Day 1 (control state) and Day 29 (test state)

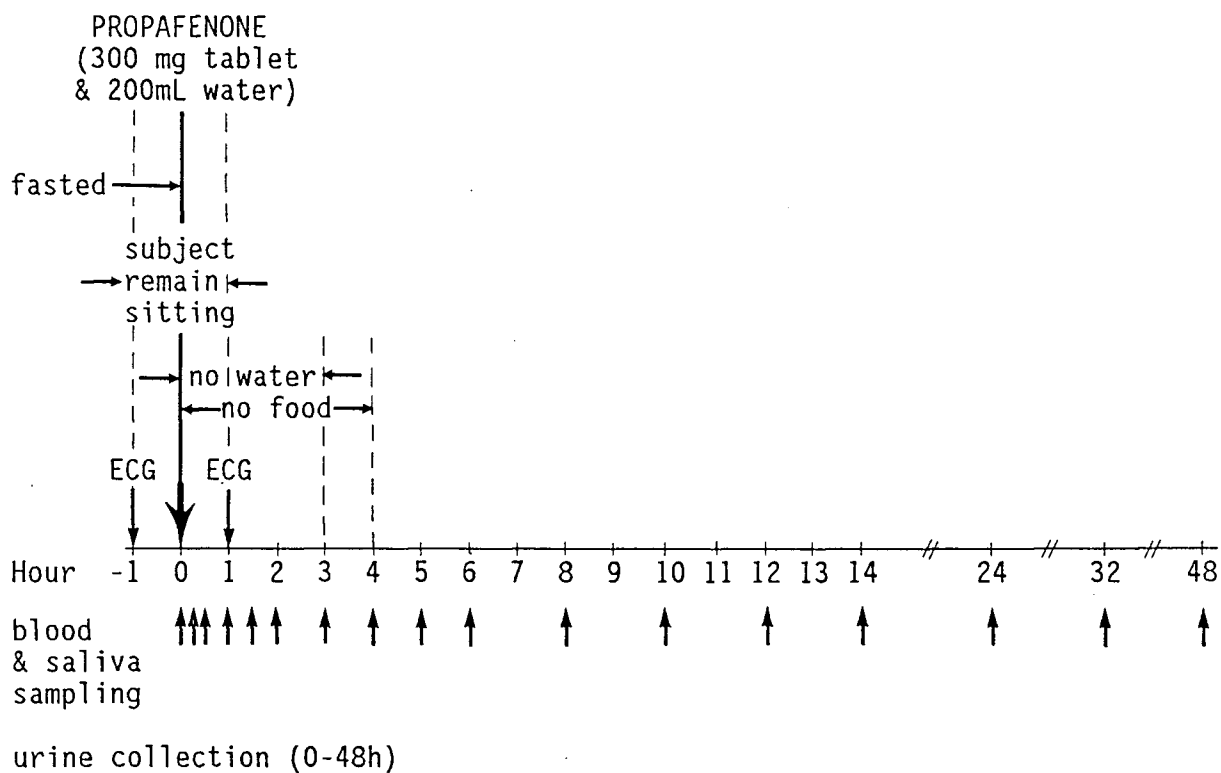


Figure 4. Scheme of study protocol.

samples were collected for 48 h. Subjects received a controlled meal for lunch and dinner (two Big Macs^R, french fries, a Sprite^R decaffeinated soft drink and an apple pie from a McDonalds^R Restaurant) on the first day of each phase of the study.

2.10.4 Sample Collection Techniques

An indwelling butterfly cannula was inserted into the brachial vein to facilitate repeated blood sampling. Blood samples (10 mL) were withdrawn from the cannula using glass syringes and 1.5 inch 22 gauge needles. One mL of saline containing heparin (5 units/mL) was injected into the cannula after the withdrawal of each sample to prevent clotting. The cannula was removed after the 14 h blood sample was collected. The 24, 32 and 48 h blood samples were drawn directly from the vein using glass syringes. Blood samples were allowed to clot at room temperature and serum obtained after centrifugation of the sample at 2,500 rpm for 20 min.

Mechanically unstimulated mixed saliva samples (2-4 mL) were collected by expectoration into a 20 mL clean glass vial at the same time as blood collection. The pH of saliva was measured immediately after collection with a pH meter. Saliva samples were centrifuged at 2,500 rpm for 10 min to remove any insoluble non-salivary substances and cellular debris; the clear supernatant was subsequently used for analysis.

Urine was collected in sterilized plastic Whirl Pak^R urine sample bags. The pH and the volume of each urine sample were immediately measured and recorded during the time period where the subjects were under supervision at the hospital. Urine from each subject was then pooled to yield 0-12, 12-24 and 24-48 h samples.

All biological samples were kept at -20°C until analyzed.

2.10.5 Assurance of Phenobarbital Compliance

Single blood samples (5 mL) were collected from all subjects on day 15, 22 and 29 (day 15, 21, 25 and 29 for some subjects) and quantitated for phenobarbital concentration to ensure compliance. The protocol required the serum phenobarbital concentration to exceed 10 $\mu\text{g/mL}$ by day 21 or 22 or the daily dose of phenobarbital would be doubled (Table 4).

2.10.6 Measurement of α_1 -Acid Glycoprotein Concentration Before and After Phenobarbital Treatment

Blood samples from day 1 and day 29 were analyzed for serum AAG concentration in all subjects to determine the effect of phenobarbital administration on AAG concentration. The procedure used is the same as in Experimental Section 2.9.8.2.

2.10.7 Analytical Procedures

2.10.7.1 Phenobarbital concentration

Serum phenobarbital concentrations were analyzed by the Division of Clinical Chemistry, Department of Pathology, University Hospital, U.B.C. Site, Vancouver, B.C. A fluorescent polarization immunoassay technique was used and the analyses were performed on an Abbott TDX, Abbott Laboratories Diagnostics Division, Irving, TX, U.S.A. The coefficient of variation was 2.2% (at a phenobarbital concentration of 10 $\mu\text{g/mL}$) and 2.8% (at a phenobarbital concentration of 52 $\mu\text{g/mL}$).

2.10.7.2 Serum and Saliva Samples

Propafenone in both serum and saliva samples and 5-hydroxy PF in

Table 4. Serum phenobarbital concentrations of non-smoking and smoking subjects during phenobarbital treatment.

Subject	Serum phenobarbital concentration ($\mu\text{g/mL}$)				
	Day 15	Day 21	Day 22	Day 25	Day 29
Non-smokers					
BK	12.3	-*	13.8	-	16.0
NP	11.0	-	15.4	-	19.2
CA	9.0	-	12.3	-	13.1
DA	10.6	-	14.5	-	16.0
SG	9.4	-	14.2	-	13.8
MV	10.4	-	14.6	-	16.8
GP	12.9	-	14.3	-	16.8
UH	7.9	15.8	-	21.6	20.2
Smokers					
JL	8.5	-	11.6	-	17.4
MA	13.0	-	18.6	-	20.4
TN	11.1	-	17.9	-	18.1
DW	11.4	-	14.6	-	16.9
GE	12.5	18.3	-	19.0	19.5
MG	9.3	12.5	-	12.5	13.0
SR	10.2	10.7	-	11.4	12.3
DB	10.9	13.9	-	15.3	15.1

* serum phenobarbital concentration not measured

serum samples were quantitated using the developed capillary GLC-ECD technique discussed in the Experimental Sections 2.5 and 2.6.

2.10.7.3 Urine Samples

Glucuronide and sulfate conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF were hydrolyzed using β -glucuronidase/arylsulfatase (Helix pomatia, Boehringer Mannheim, West Germany) [Hege *et al.*, 1984b]. Samples were first incubated with the enzyme (50 μ L per mL of urine) in a 37°C water bath at pH 4.6 for 24 h and then at pH 6.2 for another 24 h to hydrolyze 5-hydroxy PF conjugates to 5-hydroxy PF and 5-hydroxy-4-methoxy PF conjugates to 5-hydroxy-4-methoxy PF [Hege *et al.*, 1984b]. I.S.-b was then added to the samples and 5-hydroxy PF and 5-hydroxy-4-methoxy PF were extracted and quantitated using a published HPLC method [Harapat and Kates, 1982]. The absorbance was monitored at 209 nm. The HPLC method was slightly modified to obtain better resolution of I.S.-b, 5-hydroxy PF and 5-hydroxy-4-methoxy PF. The modifications include using toluene: dichloromethane:isopropyl alcohol (7:3:1) instead of 1% isoamyl alcohol in heptane as the extraction solvent and acetonitrile: phosphate buffer (pH 2.9) (20:80) as an alternative to the acetonitrile: phosphate buffer (pH 2.4) (25:75) as the mobile phase.

2.10.7.4 Protein Binding

The free fraction of PF in the 2 and 4 h serum samples obtained from each subject was estimated using the equilibrium dialysis technique described in the Experimental section 2.9.3. The serum AAG concentrations of the 0 h (blank) samples were quantitated by the RID technique described in the Experimental section 2.9.8.2.

2.10.8 Data Analysis

2.10.8.1 Propafenone Serum Data

A best fit of the serum concentration *versus* time data was obtained using the computer programme AUTOAN (Sedman and Wagner, 1976) (with equal weight of all data points) to obtain the apparent elimination rate constant describing the terminal portion of the serum drug concentration *versus* time curves (β). The apparent terminal elimination half-life ($t_{1/2\beta}$) was calculated by dividing 0.693 by β .

The area under the serum concentration *versus* time curve from time zero to infinity (AUC_0^∞) was calculated by using Equation 1:

$$\begin{aligned} AUC_0^\infty &= AUC_0^t + AUC_t^\infty \\ &= \int_0^t C_p dt + C_p \text{ last}/\beta \end{aligned} \quad (1)$$

where t represents the time of the last sample with detectable PF concentration ($C_p \text{ last}$) and β is the terminal elimination rate constant.

The area under the first moment of the concentration *versus* time curve from time zero to infinity ($AUMC_0^\infty$) was calculated by using Equation 2.

$$\begin{aligned} AUMC_0^\infty &= AUMC_0^t + AUMC_t^\infty \\ &= \int_0^t t C_p dt + t C_p \text{ last}/\beta + C_p \text{ last}/\beta^2 \end{aligned} \quad (2)$$

The CL_{int} of PF was calculated by dividing the dose by the oral AUC, assuming that CL_{int} is equal to the oral clearance (CL_o) (*i.e.*, for this completely absorbed drug and that the liver is the sole eliminating organ

that the venous equilibrium model of hepatic drug clearance applies) [Wilkinson and Shand, 1975].

Peak serum concentration (C_{\max}) and the time to reach peak serum concentration (t_{\max}) were estimated from individual's serum data.

The apparent volume of distribution (V_{darea}), using the compartment model method, was calculated using Equations 3 and 4.

For one compartment model:

$$V_{darea} = \frac{F \times D}{\beta \times AUC} = \frac{F \times CL_0}{\beta} \quad (3)$$

For two compartment model:

$$V_{darea} = \frac{F \times D}{C_{po}} \times \left(\frac{k_{12} + k_{21}}{k_{21}} + \frac{k_{13} - \beta}{k_{21}} \right) \quad (4)$$

where F = systemic availability

D = dose

C_{po} = intercept of k_a slope with ordinate

k_{12} = distribution rate constant for transfer of drug from central to peripheral compartment

k_{21} = distribution rate constant for transfer of drug from peripheral to central compartment

k_{13} = elimination rate constant of drug from central compartment

The volume of distribution at steady-state (V_{dss}), using non-compartment model method, was calculated using Equation 5 [Perrier and Mayersohn, 1982].

$$V_{dss} = \frac{F \times D}{AUC} \times \left(\frac{AUMC}{AUC} - \frac{1}{k_a} \right) \quad (5)$$

The value of F for PF is ~ 0.12 in healthy volunteers [Hollmann et al., 1983a]. Since F changed after phenobarbital treatment and the absolute values of F are unknown, all the volume of distribution terms were expressed as V_d/F .

Because of the great interindividual variability in all kinetic parameters of PF, each subject served as his own control. Kinetic parameters such as CL_{int} , C_{max} and AUC after phenobarbital treatment were compared to the control values and the changes (increase or decrease) expressed as percent of the control values.

$$\text{percent change in } CL_{int} = \frac{(CL_{int} P - CL_{int} C)}{CL_{int} C} \times 100\%$$

$$\text{percent change in } C_{max} = \frac{(C_{max} P - C_{max} C)}{C_{max} C} \times 100\%$$

$$\text{percent change in AUC} = \frac{(AUC_P - AUC_C)}{AUC_C} \times 100\%$$

where C = control (untreated)
P = phenobarbital-treated

2.10.8.2 5-Hydroxy Propafenone Serum Data

A best fit of the serum concentration *versus* time data was obtained using the computer programme AUTOAN (Sedman and Wagner, 1976) (with equal weight of all data points) to obtain β . The $t_{1/2\beta}$ was calculated by dividing 0.693 by β .

The AUC values were calculated using Equation 1. The changes

(increase or decrease) in C_{\max} and AUC after phenobarbital treatment were expressed as percentage of the control values.

The C_{\max} and t_{\max} were estimated from individual's serum data.

2.10.8.3 Propafenone Salivary Data

The saliva/serum concentration ratio of PF was calculated by dividing PF salivary concentration by the PF serum concentration determined at the identical time after drug administration. The AUC_0^t was estimated by using Equation 6 and the change (increase or decrease) in this parameter after phenobarbital treatment expressed as percentage of the control values.

$$AUC_0^t = \int_0^t C_p dt \quad (6)$$

The percent of free (unbound) PF in serum was calculated by using Equation 7 [Matin et al., 1974] (the derivation of Equation 7 is shown in Appendix 1) and compared to the values obtained from equilibrium dialysis.

Percent of free PF in serum

$$= \frac{C_{p \text{ saliva}}}{C_{p \text{ serum}}} \times \frac{1 + 10^{pK_a - 7.4}}{1 + 10^{pK_a - pH_s}} \times 100 \quad (7)$$

where pK_a = pK_a of PF
 pH_s = pH of saliva

2.10.8.4 Urine Data

The renal excretion (cumulative, 0-12, 12-24 and 24-48 h) of the conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF before and after phenobarbital treatment was calculated and expressed as percent of dose of intact drug (corrected for molecular weight difference).

2.10.9 Statistical Analysis

All kinetic parameters after phenobarbital treatment were compared to the control values using the Wilcoxon paired-sample test at the 5% level of significance.

Statistical evaluation was also performed on various pharmacokinetic parameters between non-smokers and smokers using the Mann-Whitney test with tied ranks at the 5% level of significance.

The correlation between PF serum total concentration, PF serum free concentration and PF salivary concentration was determined by linear regression.

2.11 Evaluation of Propafenone Serum Concentration-Response Relationship

2.11.1 Study Subjects and Protocol

Ten patients (aged 30-71 y) who were receiving PF for treatment of atrial fibrillation participated in the study (Table 5). All patients had normal liver and kidney function at the time of the study. They were not on any other medication(s) other than PF (mean daily oral dose = 650 mg). Serial blood samples including predose (trough level) and 4-5 postdose samples (up to 24 h) were collected. Signal-averaged ECGs (SAECGs) were obtained from each subject at each time of blood sampling using a model 1200-ETX ECG signal averager, Arrhythmia Research Technology, Houston, TX, U.S.A.

Table 5. Characteristics of patient subjects.

Patient	Age (y)	Weight (kg)	Oral dose of propafenone	Type of arrhythmia(s)
BN	71	-*	300mg q8h	A-flutter, A-fib, PAF, AT
C	68	53.3	300mg q8h	A-fib
JC	42	-	300mg bid	A-fib
JL	54	-	150mg qid	A-fib, CAD
DT	69	90.0	300mg q8h	Recurrent A-fib
LS	67	72.2	150mg tid	VPB's, VT
GB	65	-	300mg q8h	A-flutter, A-fib
RM	30	94.5	300mg q8h	A-flutter, A-fib, SVT
AP	49	-	300mg q8h	A-fib, APB's
BM	57	-	300mg q8h	A-fib, PAF

* body weight unavailable

q8h every eight hours
 bid twice a day
 qid four times a day
 tid three times a day
 A-flutter atrial flutter
 A-fib atrial fibrillation
 AT atrial tachycardia
 PAF paroxysmal atrial fibrillation
 CAD coronary artery disease
 VPB's ventricular premature beats
 VT ventricular tachycardia
 SVT supraventricular tachycardia
 APB's atrial premature beats

2.11.2 Analytical Procedures

2.11.2.1 Serum Samples

Propafenone and 5-hydroxy PF concentrations in serum samples were quantitated using the newly developed capillary GLC-ECD technique discussed in the Experimental section 2.5 and 2.6.

2.11.2.2 Protein Binding

Two samples were chosen from each subject for a protein binding study (on the basis that the PF concentrations in these samples were close to peak and trough levels). The free fraction of PF in these samples was estimated using equilibrium dialysis as described in the Experimental section 2.9.3. Serum AAG concentration of these samples were quantitated by the RID technique given in the Experimental section 2.9.8.2.

2.11.3 Measurement of Pharmacological Effect

The QRS width was used as an indicator of the pharmacological response of PF [Siddoway *et al.*, 1987]. The SAE-ECGs data was generated by averaging 150 beats and QRS widening was determined to within 1 msec by an observer not involved in this study.

2.11.4 Data Analysis

The area under the serum concentration *versus* time curve of PF over one dosing interval (AUC_0^T) at steady-state was calculated using the trapezoidal rule. CL_{int} was calculated by dividing the dose by AUC_0^T . In four patients (JC, JL, DT and LS), the predose PF serum concentration was

used as the PF serum concentration at the dosing interval in the estimation of AUC_0^T and CL_{int} . The steady-state serum concentration of PF (C_{pss}) was calculated by dividing AUC_0^T by the dosing interval (τ).

The area under the serum concentration *versus* time curve of 5-hydroxy PF over one dosing interval was also calculated using the trapezoidal rule.

2.11.5 Statistical Analysis

Linear regression was used to obtain the correlation between PF serum concentration and QRS width and between 5-hydroxy PF serum concentration and QRS width. For the samples for which protein binding was performed, linear regression was also used to obtain the correlation between PF serum total concentration and QRS width and, as well, PF serum free concentration and QRS width.

Multiple stepwise regression [computer program RS-1 (Bolt, Baranek and Newman Software Products Corporation, Cambridge, MA, U.S.A.)] was used to obtain an equation to predict QRS width as a function of PF serum concentration, 5-hydroxy PF serum concentration and serum AAG concentration.

3. RESULTS

3.1 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of Propafenone

3.1.1 Preliminary Results of the Capillary GLC-ECD Method of Analysis for Propafenone

3.1.1.1 Extraction and TFAA Treatment

TFAA has been reported to form highly electron withdrawing derivatives with compounds possessing reactive hydroxyl and/or amino groups. Derivatization of PF and the I.S.-a with TFAA yielded two sharp and completely resolved peaks on the GLC chromatogram. However, the occasional presence of an unresolved negative peak before the I.S.-a peak (Figure 5) caused a potential error in peak area integration making a TFAA derivative inapplicable with this column. This problem was solved by using other acylating agents such as PFPA or HFBA. The sensitivity was further improved when HFBA, which contains four more fluorine atoms than TFAA, was employed (HFBA yielded an approximately five-fold increase in sensitivity over TFAA).

3.1.1.2 Neutralization of Excess Derivatizing Agent

The appearance of extra peaks in the GLC chromatogram (Figure 6) suggested that the removal of excess HFBA by hydrolysis with water and subsequent neutralization with ammonium hydroxide caused a rapid decomposition of the HFB esters (HFB acylation of the hydroxyl group) of PF and I.S.-a by alkali hydrolysis.

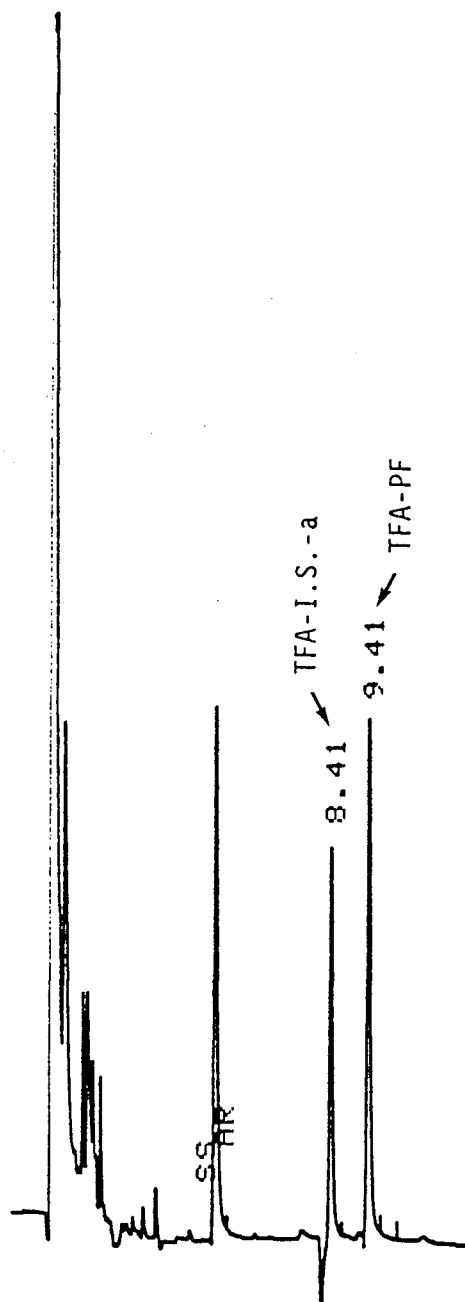


Figure 5. Chromatogram of the TFA derivatives of propafenone and I.S.-a.

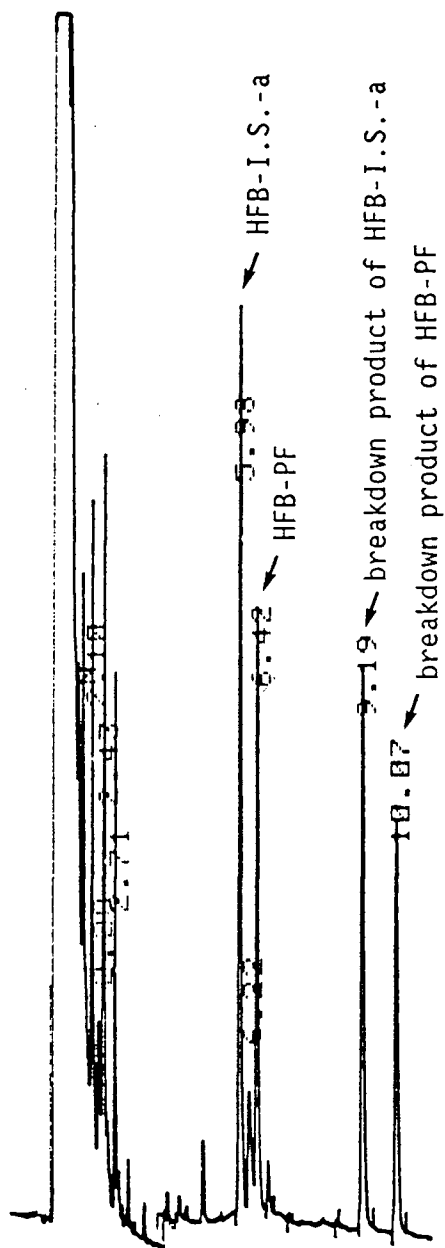


Figure 6. Chromatogram of the HFB derivatives of propafenone and I.S.-a.

Evaporation, a widely accepted technique [Walle and Ehrsson, 1971], was used to remove excess HFBA. In this method, the sample was allowed to cool to room temperature after the derivatization reaction and then dried under a gentle stream of nitrogen in a 35°C water bath. The residue was reconstituted with toluene and injected into the GLC.

Another alternative method was to neutralize the sample with 0.5 mL of phosphate buffer (pH 6) for 30 sec [Ehrsson *et al.*, 1971]. The toluene layer was then transferred and diluted with toluene to a desired volume and injected into the GLC. This method was later employed as part of a routine procedure for the analysis of PF.

3.1.2 Optimal Derivatization Conditions

Data illustrating the need for using TEA as a catalyst and determination of the optimum reaction time are shown in Figure 7. No significant difference was observed in the peak area of PF between 0.5 and 4 h when TEA was used, indicating the reaction was maximal in 0.5 h. When TEA was not incorporated into the reaction mixture, the derivatization was slow and incomplete, as illustrated by the slow rise in the curve and lack of attainment of a plateau. A further study with incubation times of 0, 5, 15, 30, 45 and 60 min indicated that the reaction was complete within 15 min in the presence of TEA (Figure 8), thus permitting the use of a shorter incubation time. The minimum amount of TEA (0.003 M) required was 400 μ L (Figure 9) and the minimum amount of HFBA required was 20 μ L (Figure 10).

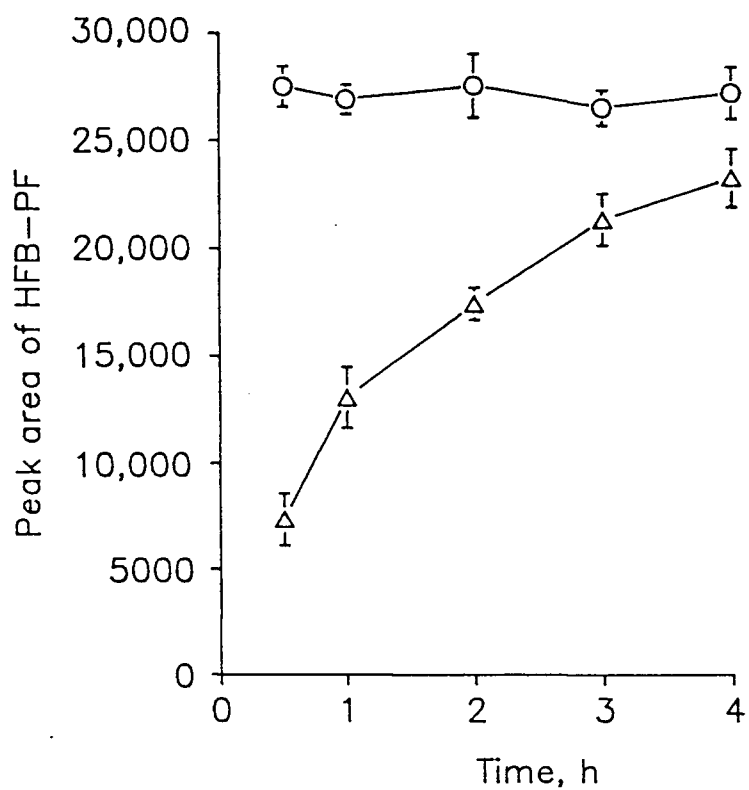


Figure 7. Effect of TEA on the HFB-PF response, as estimated by the peak area of HFB-PF. Samples were derivatized with HFBA and incubated at 65°C for various time with (O) or without (Δ) TEA. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

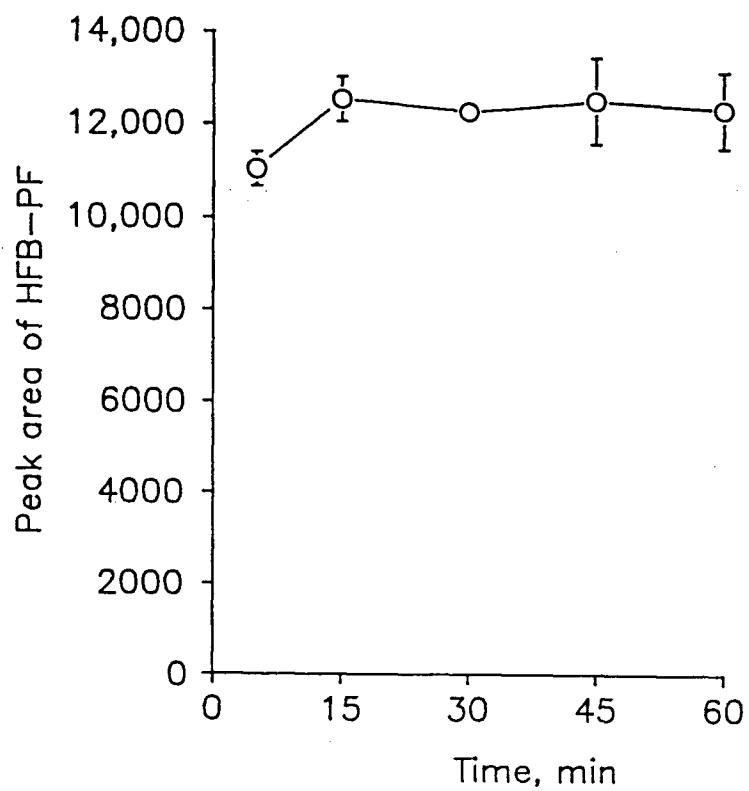


Figure 8. Effect of derivatization time on the HFB-PF response, as estimated by the peak area of HFB-PF. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

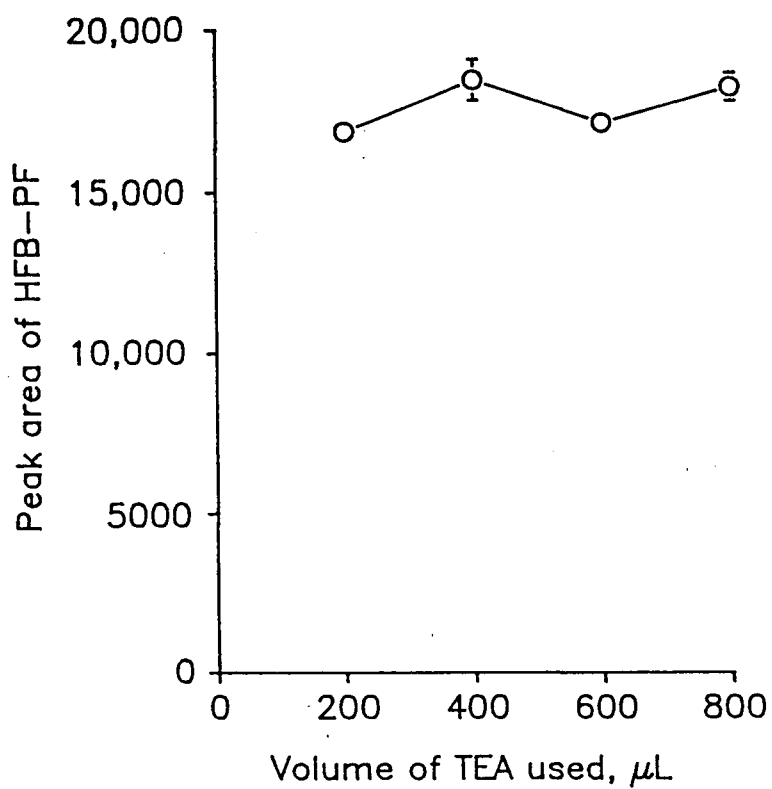


Figure 9. Effect of the volume of TEA (0.003M in toluene) on the HFB-PF response, as estimated by the peak area of HFB-PF. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

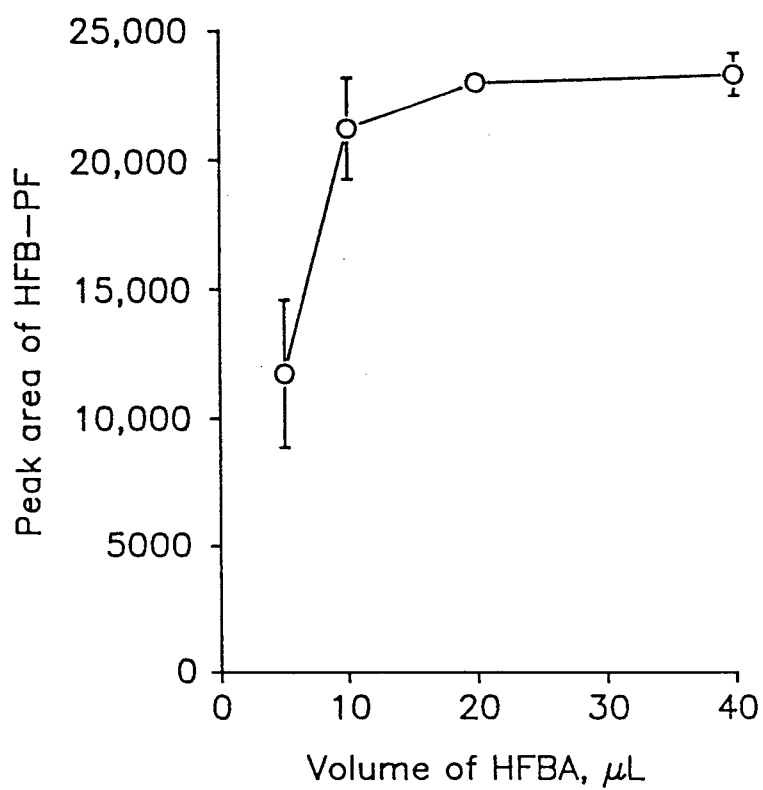


Figure 10. Effect of the volume of HFBA on the HFB-PF response, as estimated by the peak area of HFB-PF. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

3.1.3 Extraction Efficiency of Solvents

The extraction efficiency (E) of solvents was tested by measuring the maximal peak area obtained for the chromatographic peak of HFB-PF as a function of the solvent system used. The results are shown in Figure 11. Benzene was found to provide optimal extraction of PF ($E = 0.81$) with toluene showing slightly diminished efficiency ($E = 0.76$). Neither the less polar solvent, hexane, ($E = 0.49$) nor the slightly more polar solvent combination of toluene:dichloromethane:isopropyl alcohol (7:3:1) ($E = 0.45$) gave satisfactory results.

3.1.4 Optimal GLC-ECD Conditions

The inlet purge activation time (~60 sec, Figure 12) controls sample transfer to the column during splitless injection with minimal loss of solute ($\leq 1\%$) and sufficient removal of solvent (~5%) to reduce tailing. No significant difference in the peak area ratio (HFB-PF/HFB-I.S.-a) was observed between the different injection port temperatures tested (200-260°C, Figure 13A). However, peak broadening occurred when the injection port temperature was lowered to 200°C. There were also no significant differences in the response between the detector temperatures tested (330-360°C, Figure 13B) and between the various make-up gas flow rates tested (30-60 mL/min, Figure 13C). The optimal temperature programming conditions were: initial column temperature, 220°C; rate, 4°C/min; final column temperature, 270°C; injection port temperature, 210°C; ECD temperature, 350°C and make-up gas flow rate, 60 mL/min.

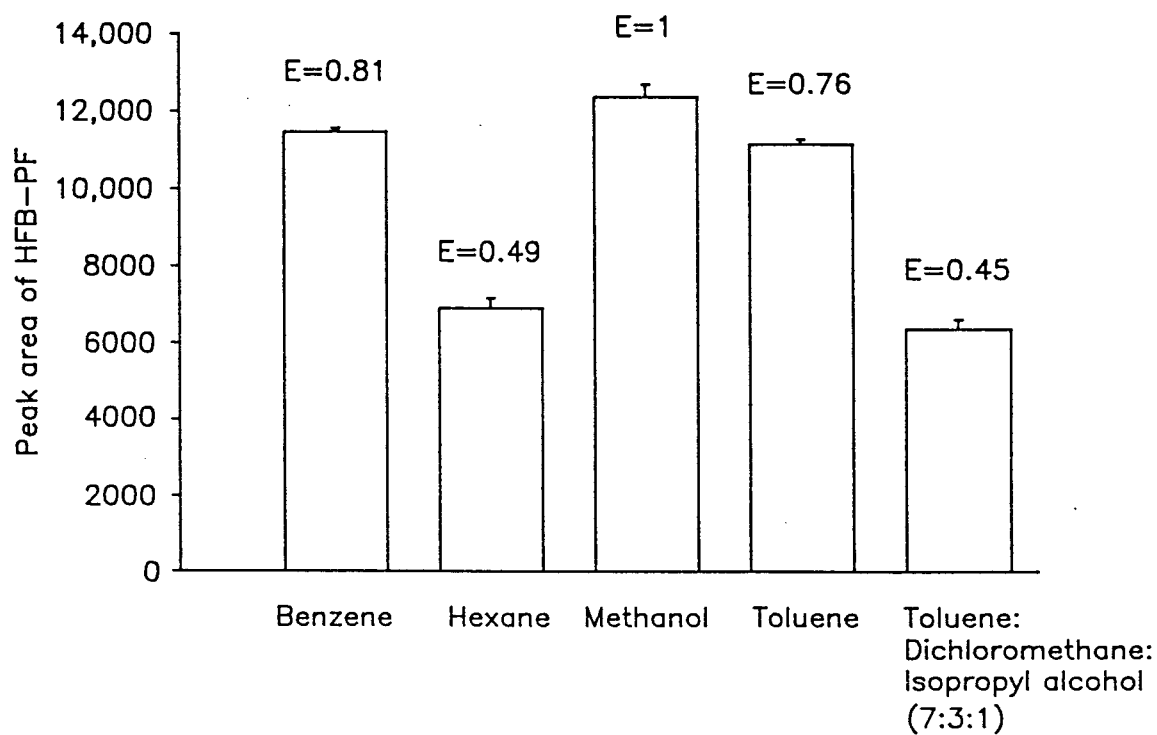


Figure 11. The extractability of propafenone from serum using different solvents. The extraction efficiency (E) of each solvent was estimated as compared to methanol (unextracted, $E = 1$). Number of samples = 3, duplicate injections.

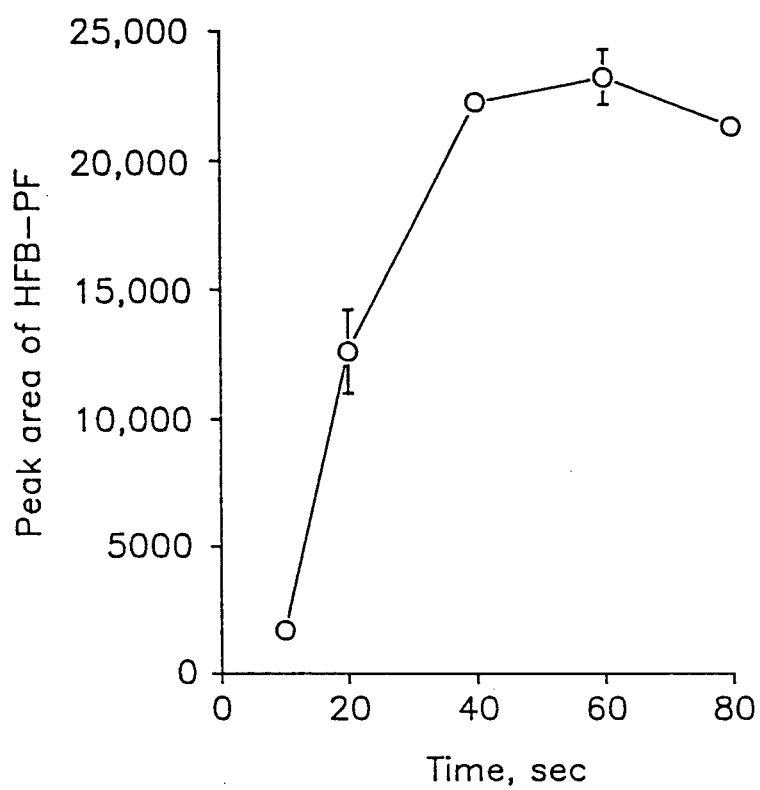


Figure 12. Effect of inlet purge valve activation time on the HFB-PF response, as estimated by the peak area of HFB-PF. The data are presented as mean \pm 1s.d.

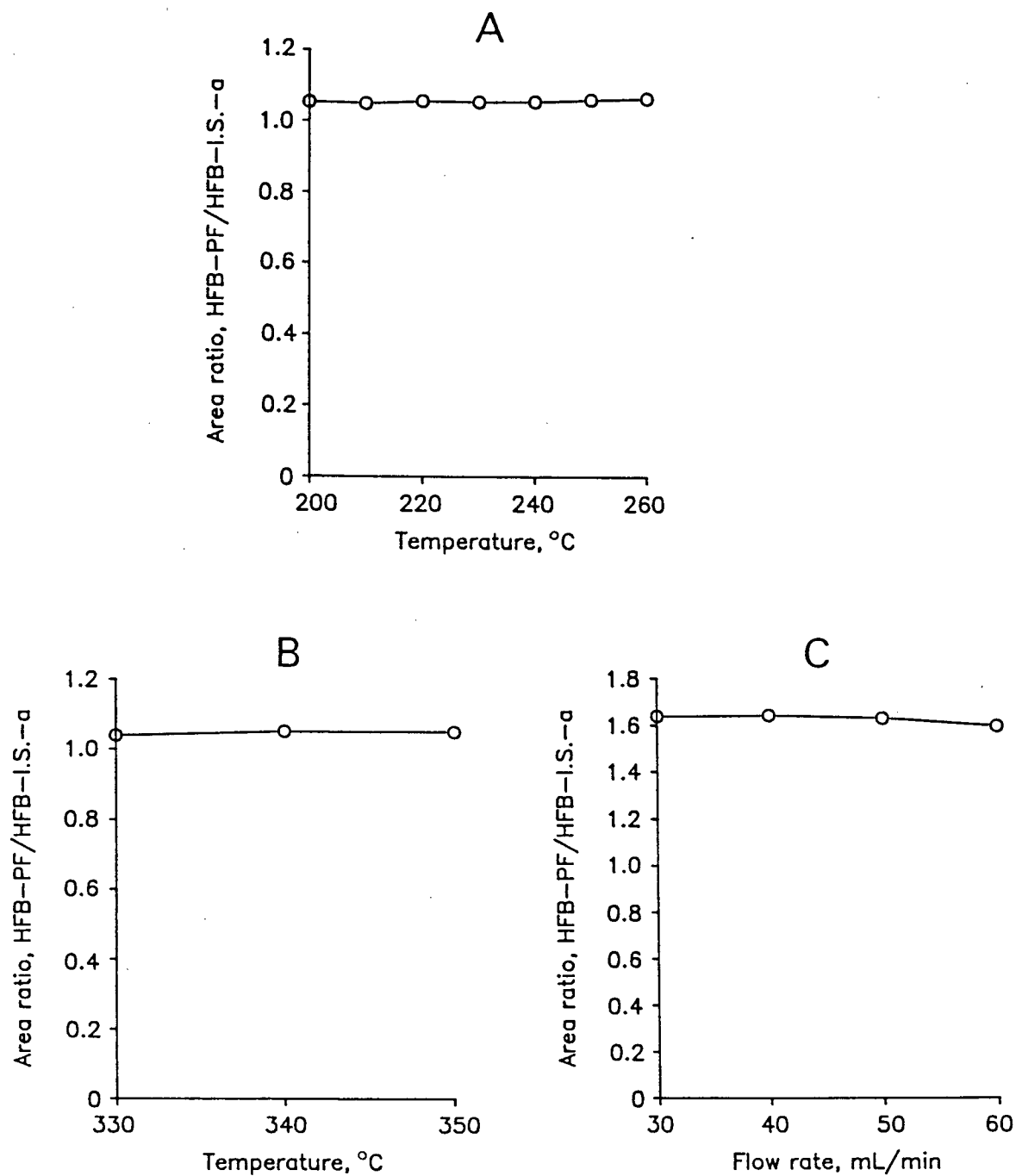


Figure 13. Effect of (A) injection port temperature, (B) detector temperature and (C) make-up gas flow rate on the HFB-PF response, as estimated by the peak area ratio (HFB-PF/HFB-I.S.-a). The data are presented as mean \pm 1s.d.

3.1.5 Recovery of Propafenone

3.1.5.1 Extractability of Propafenone

The recovery of PF from plasma after solvent extraction is shown in Table 6 and was found to be approximately 90% over the concentration range of 10-100 ng/mL.

3.1.5.2 Variability in Recovery of Propafenone

A significant linear relationship was demonstrated between the actual and the calculated amounts of PF obtained during the course of a recovery experiment (Figure 14). The best fit through the data points was obtained by linear regression and was described by $Y = 0.838X - 0.962$ with a correlation coefficient (r) of 0.995.

3.1.6 Variability Test

The day-to-day variability test showed that the coefficient of variation was 0.5-4.5% and 4.5% for within-run (repeatability) and between-run (reproducibility) precision, respectively. The extracted and derivatized samples were stable for up to one week without any apparent degradation when stored at -20°C .

3.1.7 Calibration Curves

The data for a representative calibration curve used in the quantitation of PF for single dose volunteer saliva and serum samples and steady-state patient serum samples are presented in Table 7. Linearity was observed over the concentration ranges studied (0.5-10 ng/mL for volunteer saliva samples, 2.5-50 ng/mL for volunteer plasma samples and 10-100 ng/mL

Table 6. Extractability of propafenone.

Number of samples, n = 3 (two injections for each sample)

Propafenone added (ng/mL)	Propafenone measured (ng/mL) (mean \pm s.d.)	Recovery (%) (mean \pm s.d.)
10	8.9 \pm 1.6	89.0 \pm 16.0
20	18.5 \pm 1.0	92.5 \pm 5.0
40	37.1 \pm 1.7	92.8 \pm 4.2
60	54.4 \pm 0.7	90.7 \pm 1.1
80	70.7 \pm 3.7	88.4 \pm 4.6
100	84.7 \pm 3.9	84.7 \pm 3.9
Mean \pm s.d.		89.7 \pm 3.0

s.d. standard deviation

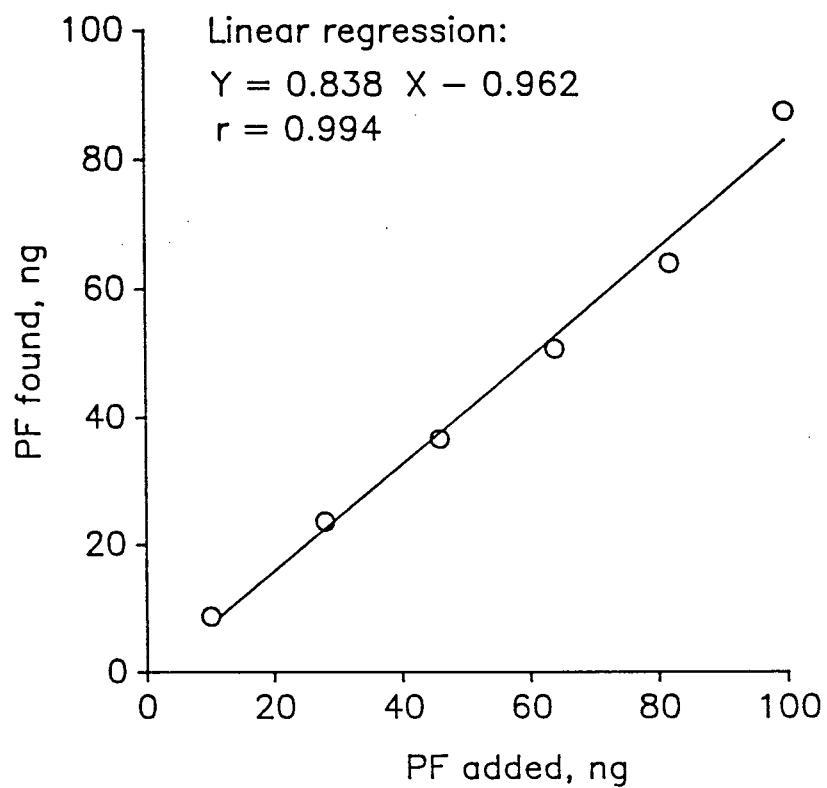


Figure 14. Variability in recovery of propafenone.

Table 7. Calibration curve data of propafenone for volunteer saliva and serum samples and patient serum samples.

Number of samples, $n = 2$ (two injections for each sample)

Concentration of propafenone (ng/mL)	Peak area ratio propafenone/I.S.-a (mean \pm s.d.)	Coefficient of variation (%)
Volunteer saliva samples		
0.5	0.067 \pm 0.004	5.8
1.0	0.138 \pm 0.005	3.7
2.0	0.267 \pm 0.008	3.0
4.0	0.511 \pm 0.006	1.2
6.0	0.775 \pm 0.017	2.2
8.0	1.016 \pm 0.019	1.9
10.0	1.269 \pm 0.073	5.8

Statistics: linear regression, $Y = 0.126X + 0.010$; $r = 0.999$

Volunteer serum samples

2.6	0.097 \pm 0.002	1.8
5.1	0.171 \pm 0.001	0.2
10.2	0.313 \pm 0.027	8.7
20.4	0.581 \pm 0.005	0.9
30.6	0.827 \pm 0.004	0.5
40.8	1.003 \pm 0.014	1.4
51.0	1.335 \pm 0.042	3.2

Statistics: linear regression, $Y = 0.025X + 0.050$; $r = 0.998$

Patient serum samples

10.1	0.191 \pm 0.002	1.1
20.3	0.349 \pm 0.003	0.9
40.5	0.610 \pm 0.002	0.4
60.8	0.866 \pm 0.006	0.7
81.0	1.131 \pm 0.029	2.5
101.3	1.334 \pm 0.016	1.2

Statistics: linear regression, $Y = 0.013X + 0.088$; $r = 0.999$

s.d. standard deviation

for patient plasma samples). The best fit through the data points was obtained by linear regression and was described by $Y = 0.126X + 0.010$ with a r of 0.999, $Y = 0.025X + 0.050$ with a r of 0.998 and $Y = 0.013X + 0.088$ with a r of 0.999, respectively. The coefficient of variation (C.V.) was less than 10% over the concentration ranges studied.

3.1.8 Summary of the Extraction, Derivatization Procedure and the Resulting Chromatograms for Propafenone

The extraction and derivatization procedure for PF is shown in Figure 15. Figure 16 illustrates the proposed reaction of PF and I.S.-a with HFBA to yield the respective HFB derivatives. Figure 17A and B show representative gas chromatograms from the extracts of blank serum and blank serum spiked with PF and I.S.-a and Figure 17C shows the chromatogram of a serum sample (spiked with I.S.-a) from a subject receiving PF. The HFB derivative of PF was completely resolved from the HFB derivative of the I.S.-a. No interfering peaks were observed from endogenous serum components of the serum extract (Figure 17A) and an analysis time of less than 15 min was obtained.

3.2 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of 5-Hydroxy Propafenone and 5-Hydroxy-4-Methoxy Propafenone

3.2.1 Optimal Derivatization Conditions

The minimum amount of HFBA required for the derivatization of 5-hydroxy PF was 20 μ L (Figure 18) and the optimal incubation time was 30 min (Figure 19).

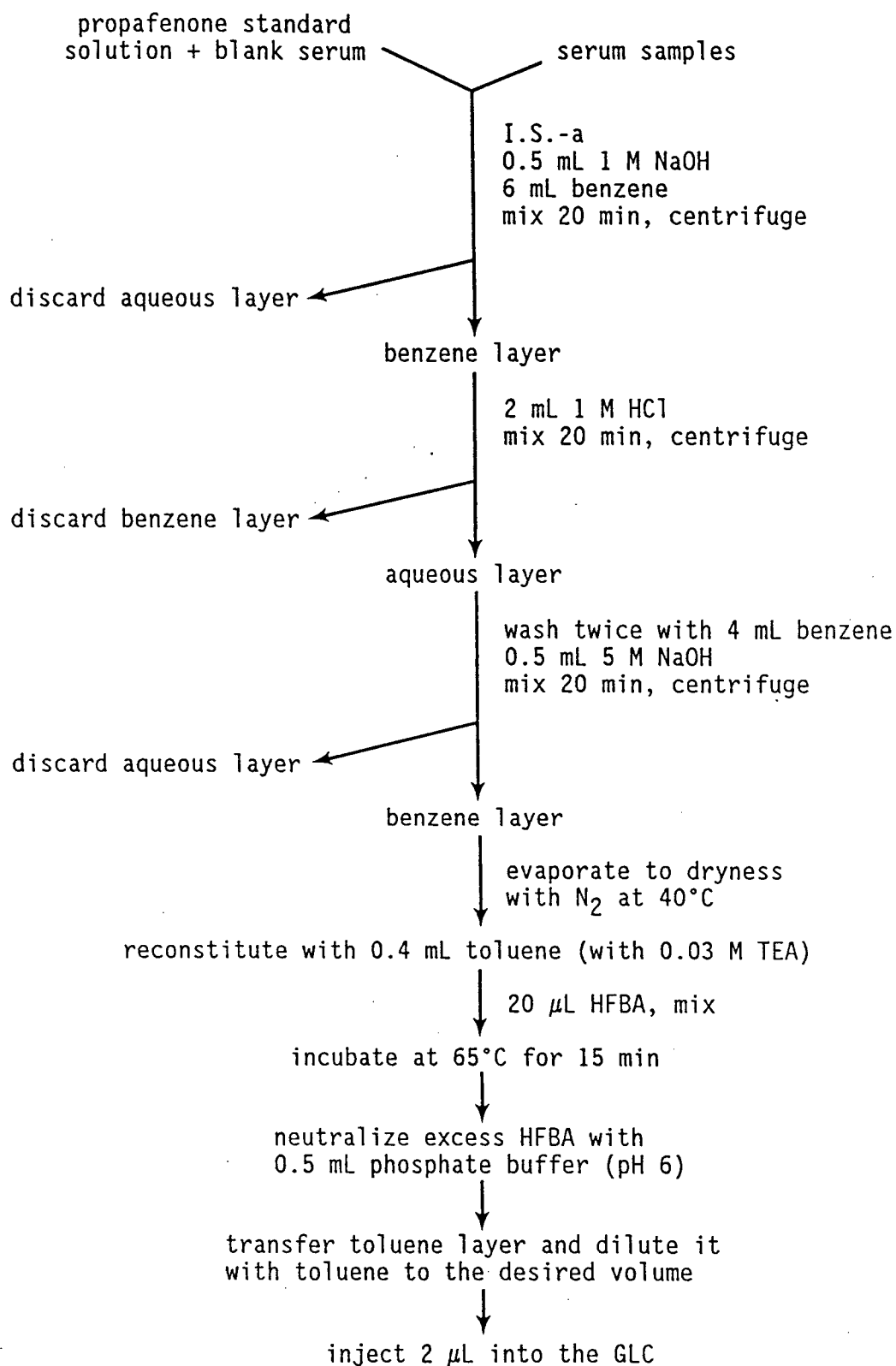
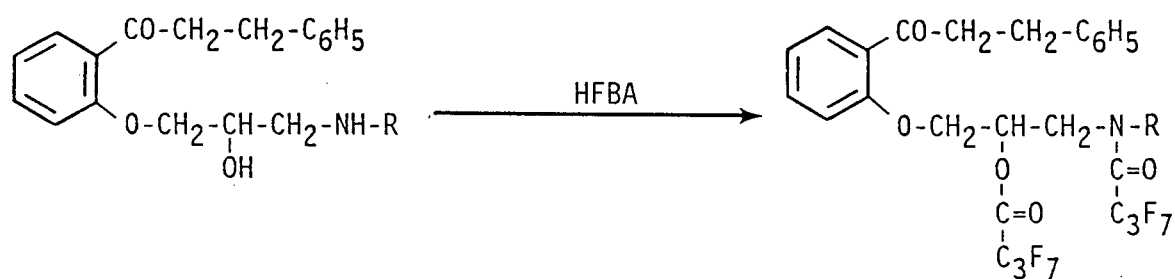


Figure 15. Scheme of extraction procedure for propafenone



propafenone $\text{R} = \text{C}_3\text{H}_7$
 I.S.-a $\text{R} = \text{C}_2\text{H}_5$

Figure 16: Derivatization of propafenone and I.S.-a to yield the HFB derivatives of propafenone and I.S.-a, respectively.

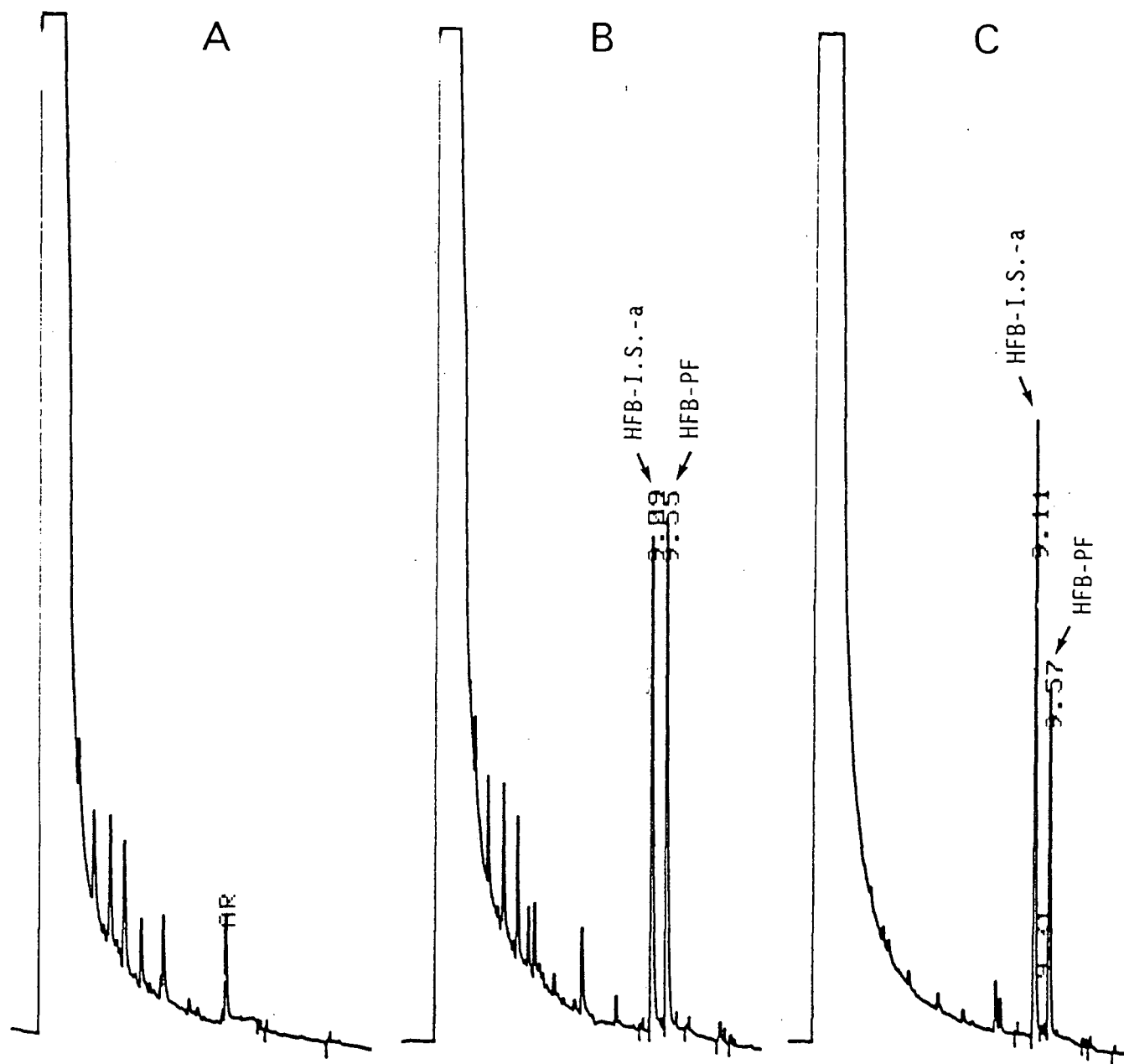


Figure 17. Chromatograms of extracts from (A) blank serum; (B) blank serum spiked with propafenone ($0.08 \mu\text{g}$) and I.S.-a ($0.07 \mu\text{g}$) and (C) a serum sample (spiked with I.S.-a, $0.07 \mu\text{g}$) from a subject receiving propafenone.

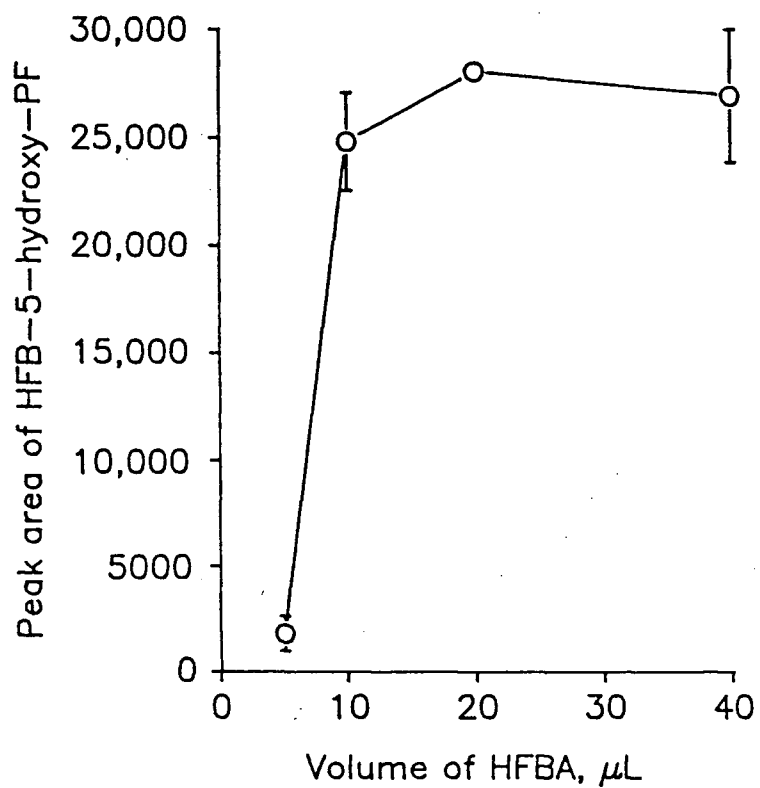


Figure 18. Effect of the volume of HFBA on the HFB-5-hydroxy PF response, as estimated by the peak area of HFB-5-hydroxy PF. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

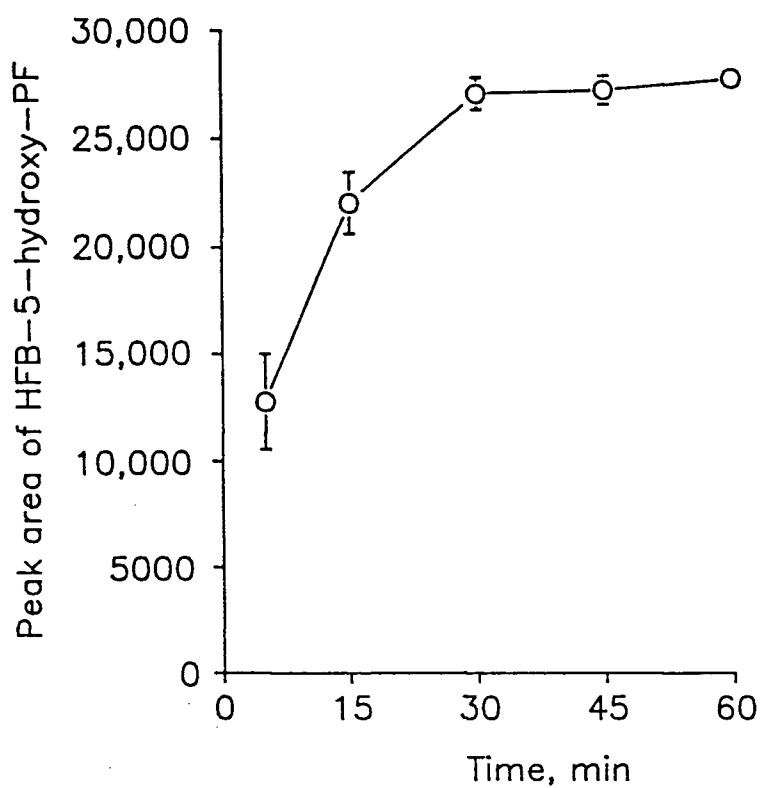


Figure 19. Effect of derivatization time on the HFB-5-hydroxy PF detector response, as estimated by the peak area of HFB-5-hydroxy PF. Number of samples = 3, duplicate injections. The data are presented as mean \pm 1s.d.

3.2.2 Extraction Efficiency of Solvents

The optimal extraction solvent for recovery of 5-hydroxy PF from serum was tested and the results shown in Figure 20. The more polar solvent mixture - toluene: dichloromethane:isopropyl alcohol (7:3:1) [Brode *et al.*, 1984] was found to provide optimal extraction for 5-hydroxy PF ($E = 0.80$). This was followed by the non-polar solvents, benzene ($E = 0.70$) and toluene ($E = 0.60$). The non-polar solvent hexane gave a very poor extraction efficiency ($E = 0.01$).

3.2.3 Optimal GLC-ECD Conditions

The optimal temperature programming conditions for routine analysis of 5-hydroxy PF were: initial column temperature, 205°C (for 0.8 min); rate, 3°C/min; final column temperature, 270°C; injection port temperature, 210°C; electron-capture detector temperature, 350°C and make-up gas flow rate, 60 mL/min.

3.2.4 Extractability of 5-Hydroxy Propafenone

The recovery of 5-hydroxy PF, following solvent extraction from serum, is shown in Table 8 and was found to be approximately 88% over the concentration range of 10-50 ng/mL.

3.2.5 Calibration Curve

The data for a representative calibration curve used in the quantitation of 5-hydroxy PF for single dose volunteer serum samples are presented in Table 9. Linearity was observed over the concentration range of 2.5-50 ng/mL. The best fit through the data points was obtained by linear regression and was described by $Y = 0.023X + 0.052$ with a r of

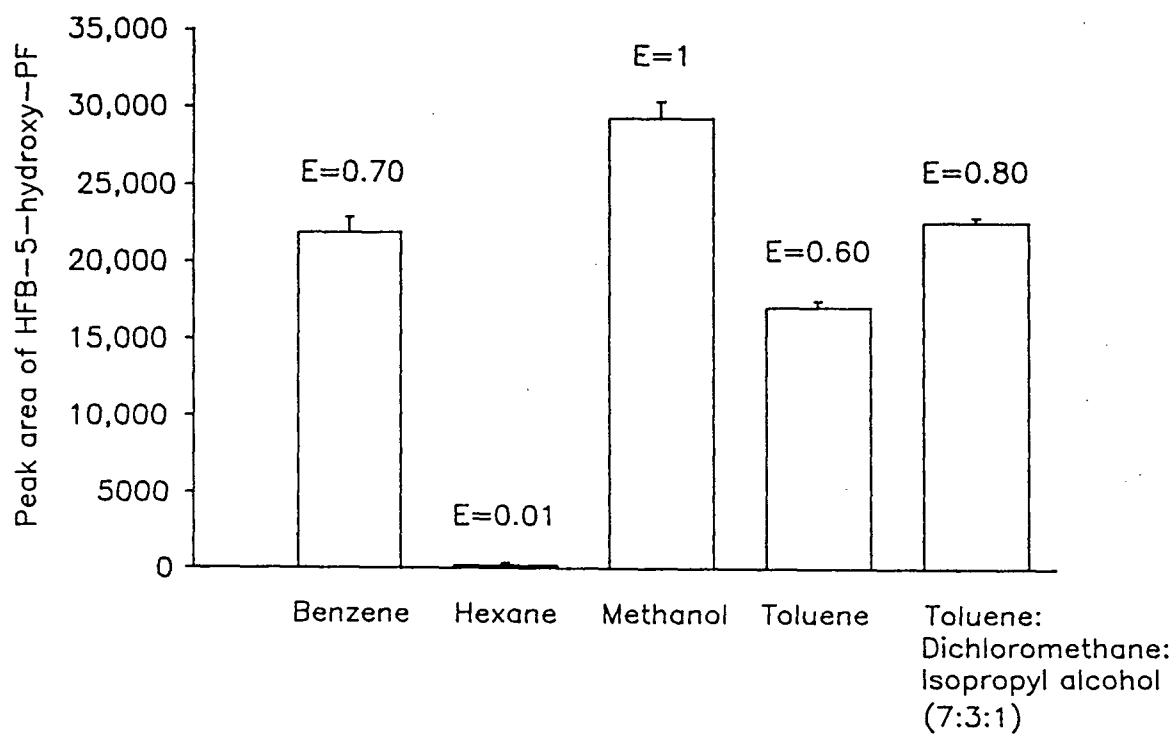


Figure 20. The extractability of 5-hydroxy propafenone from serum using different solvents. The extraction efficiency (E) of each solvent was estimated as compared to methanol (unextracted, E = 1). Number of samples = 3, duplicate injections.

Table 8. Extractability of 5-hydroxy propafenone.

Number of samples, n = 3 (two injections for each sample)

5-Hydroxy propafenone added (ng/mL)	5-Hydroxy propafenone measured (ng/mL) (mean \pm s.d.)	Recovery (%) (mean \pm s.d.)
10.9	9.3 \pm 1.0	85.7 \pm 9.4
21.8	20.0 \pm 0.6	91.9 \pm 2.9
32.7	29.6 \pm 0.8	90.5 \pm 2.4
43.6	37.3 \pm 1.1	85.6 \pm 2.5
54.4	45.6 \pm 2.3	83.8 \pm 4.2
Mean \pm s.d.		87.5 \pm 3.5

s.d. standard deviation

Table 9. Calibration curve data of 5-hydroxy propafenone.

Number of samples, n=2 (two injections for each sample)

Concentration of 5-hydroxy propafenone (ng/mL)	Peak area ratio 5-hydroxy propafenone/I.S.-b (mean \pm s.d.)	Coefficient of variation (%)
2.5	0.0743 \pm 0.0017	2.2
5.0	0.1267 \pm 0.0021	1.7
10.0	0.3136 \pm 0.0083	2.7
20.0	0.5549 \pm 0.0143	2.6
30.0	0.7678 \pm 0.0137	1.8
40.0	0.9748 \pm 0.0115	1.2
50.0	1.1468 \pm 0.0350	3.1

Statistics: linear regression, $Y=0.023X + 0.052$; $r=0.996$

s.d. standard deviation

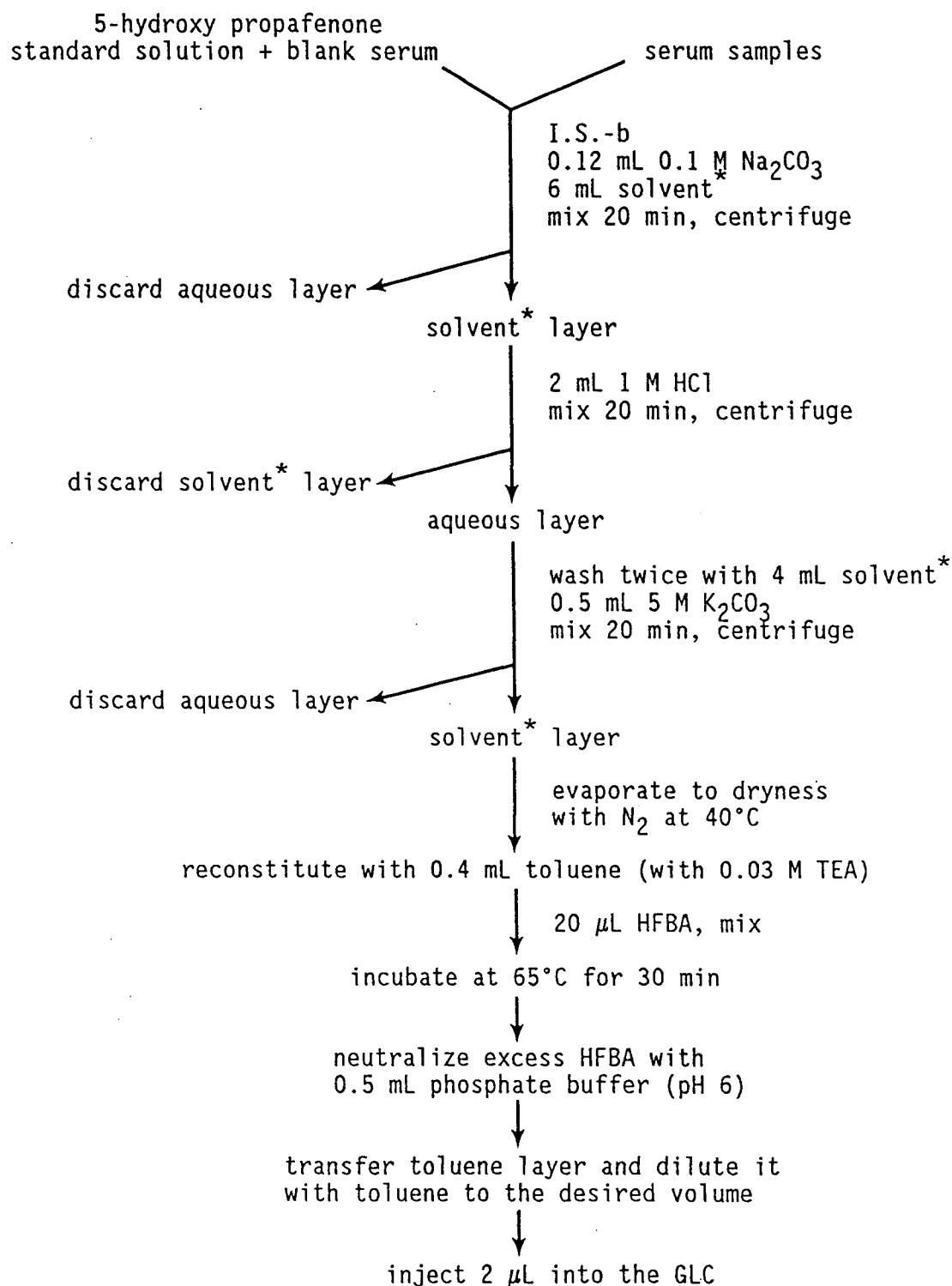
0.996. The C.V. was less than 3.1% over the concentration range studied.

3.2.6 Summary of Extraction, Derivatization Procedure and the Resulting Chromatograms for 5-Hydroxy Propafenone

The scheme of the extraction and derivatization procedure for 5-hydroxy PF is shown in Figure 21. It is similar to the extraction procedure for PF with only slight modification. A solvent mixture [toluene:dichloromethane:isopropyl alcohol (7:3:1)] was used to replace benzene as the extraction solvent since this solvent mixture is more polar and was found to give a higher extraction efficiency. Sodium and potassium carbonate were used to replace sodium hydroxide during the extraction to minimize the formation of a water-soluble sodium salt of 5-hydroxy PF which would be lost in the aqueous layer during solvent extraction. Figure 22 illustrates the proposed reaction of 5-hydroxy PF and Li-1548 with HFBA to yield the respective HFB derivatives. Figure 23A shows the chromatogram of an extracted blank serum spiked with 5-hydroxy PF and I.S.-b. Figure 23B presents the chromatogram of a serum sample (spiked with I.S.-b) from a subject receiving PF. The HFB derivatives of PF, 5-hydroxy PF and I.S.-b were completely resolved from each other.

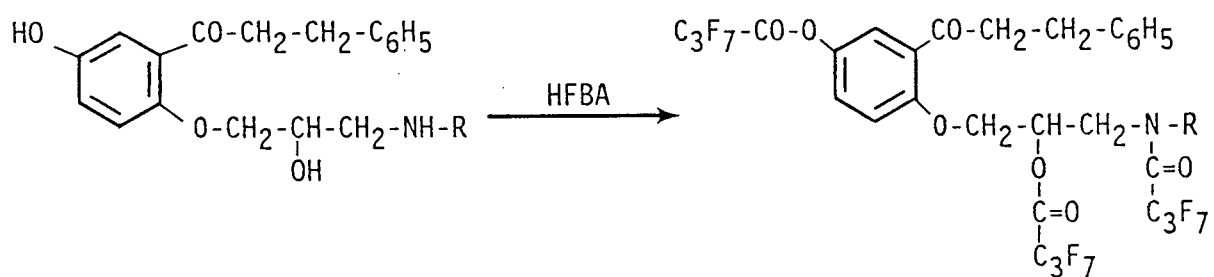
3.2.7 5-Hydroxy-4-Methoxy Propafenone

Figure 24 shows a chromatogram of the HFB derivative of 5-hydroxy-4-methoxy PF. The appearance of two peaks indicate incomplete derivatization. The derivatization was not completed even when a higher incubation temperature (up to 100°C) and/or a larger amount of HFBA (up to 200 μ L) were used.



* toluene:dichloromethane:isopropyl alcohol (7:3:1)

Figure 21. Scheme of extraction procedure for 5-hydroxy propafenone



5-hydroxy propafenone R = C₃H₇
 I.S.-a R = C₂H₅

Figure 22: Derivatization of 5-hydroxy propafenone and I.S.-b to yield the HFB derivatives of 5-hydroxy propafenone and I.S.-b, respectively.

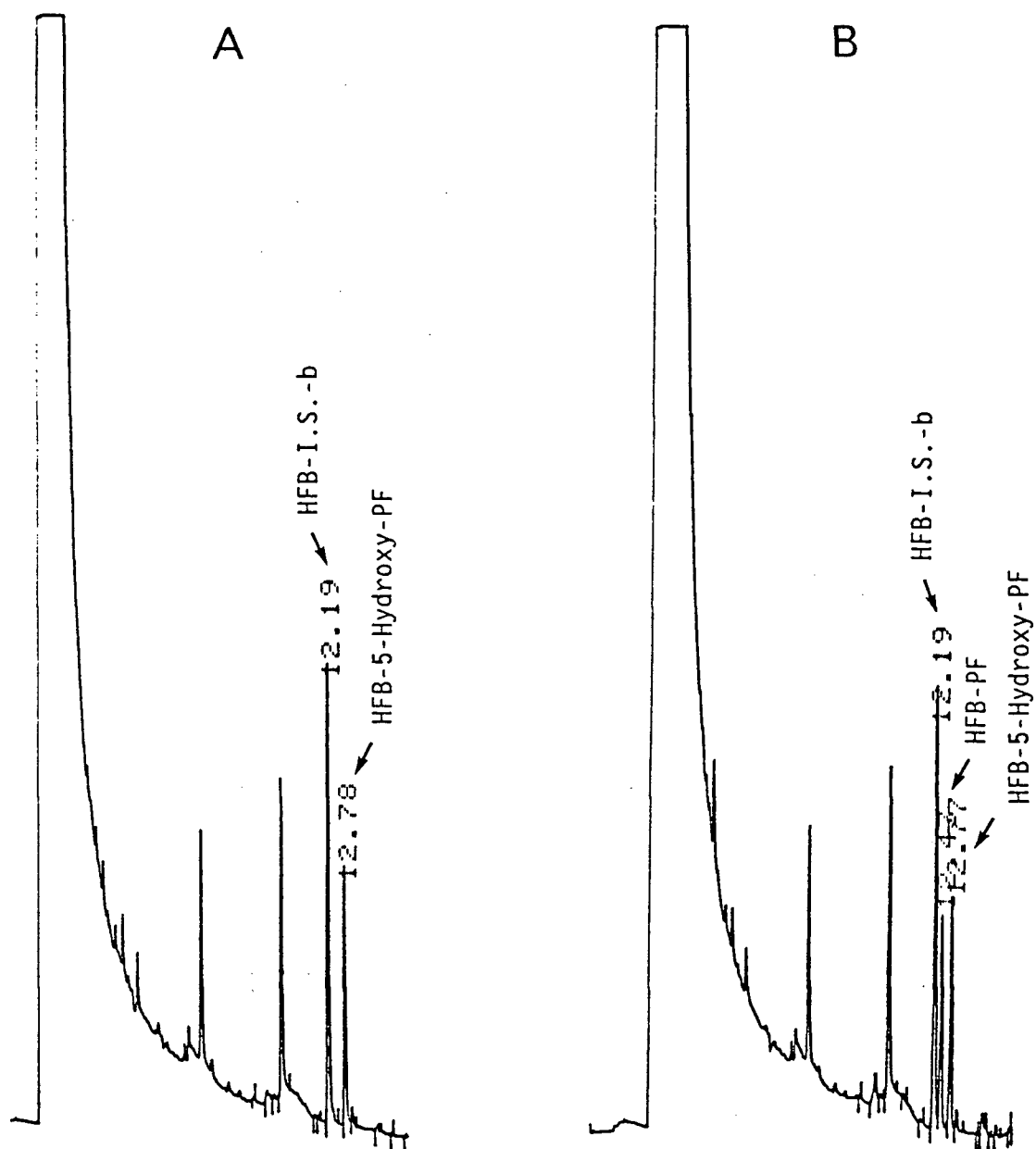


Figure 23. Chromatograms of extracts from (A) blank serum spiked with 5-hydroxy propafenone (0.02 μg) and I.S.-b (0.08 μg) and (B) a serum sample (spiked with I.S.-b, 0.08 μg) from a subject receiving propafenone.

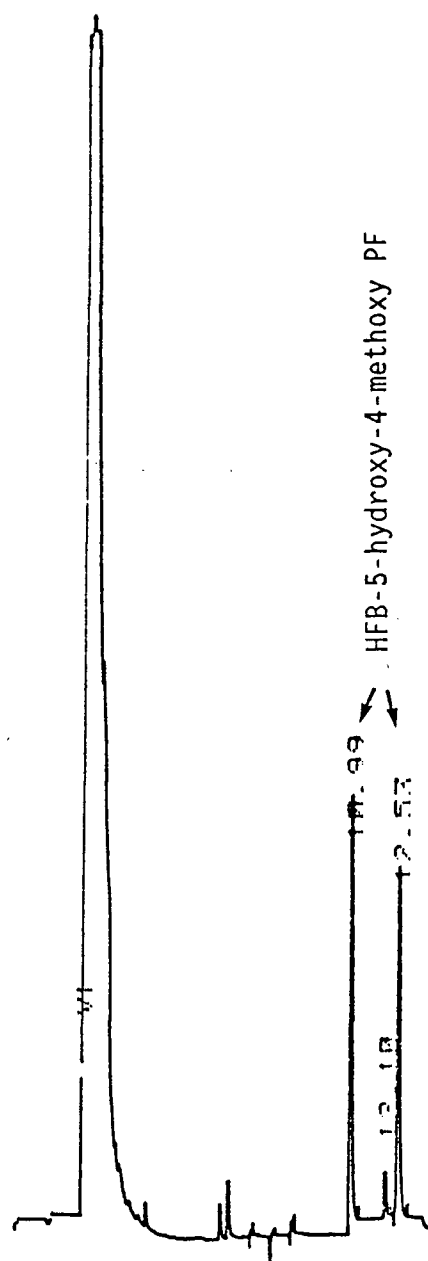


Figure 24. Chromatogram of extracts from blank serum spiked with 5-hydroxy-4-methoxy propafenone.

3.3 Structural Confirmation of HFB Derivatives of Propafenone, 5-Hydroxy Propafenone and I.S.-a by Gas-Liquid Chromatography-Mass Spectrometric Analysis.

Figures 25-29 provides the GLC-MS results including total ion chromatograms, fragmentation patterns and MS spectrum of the HFB derivative of PF with EI (Figures 25-26), PICI (Figures 27-28) and NICI (Figure 29) as the ionization modes, respectively. For EI, the molecular ion (M^+) of HFB-PF was at m/e 733 and in order of decreasing intensity, the prominent fragment ions were at m/e 91, 121, 508, 43, 104, 252, 226, 294. For PICI, the pseudomolecular ion $(M+1)^+$ for HFB-PF was at m/e 734 and in order of decreasing intensity, the prominent fragment ions were at m/e 508, 734, 520, 265, 294, 133, 252. For NICI, the pseudomolecular ion $(M-1)^-$ for HFB-PF was at m/e 732 and in order of decreasing intensity, the prominent fragment ions were at m/e 488, 213, 693, 194, 673, 448, 653.

Figures 30-32 provides the GLC-MS results including total ion chromatograms, selective fragmentation structures and MS spectrum of the HFB derivative of I.S.-a with EI (Figure 30), PICI (Figure 31) and NICI (Figure 32) as the ionization modes, respectively. For EI, the molecular ion (M^+) for HFB-I.S.-a was at m/e 719 and in order of decreasing intensity, the prominent fragment ions were at m/e 91, 494, 169, 121, 226, 280, 104. For PICI, the pseudomolecular ion $(M+1)^+$ for HFB-I.S.-a was at m/e 720 and in order of decreasing intensity, the prominent fragment ions were at m/e 494, 720, 505, 265, 288. For NICI, the pseudomolecular ion $(M-1)^-$ for HFB-I.S.-a was at m/e 718 and in order of decreasing intensity, the prominent fragment ions were at m/e 474, 213, 194, 679, 434, 659.

Figures 33-34 shows the GLC-MS-EI results including total ion

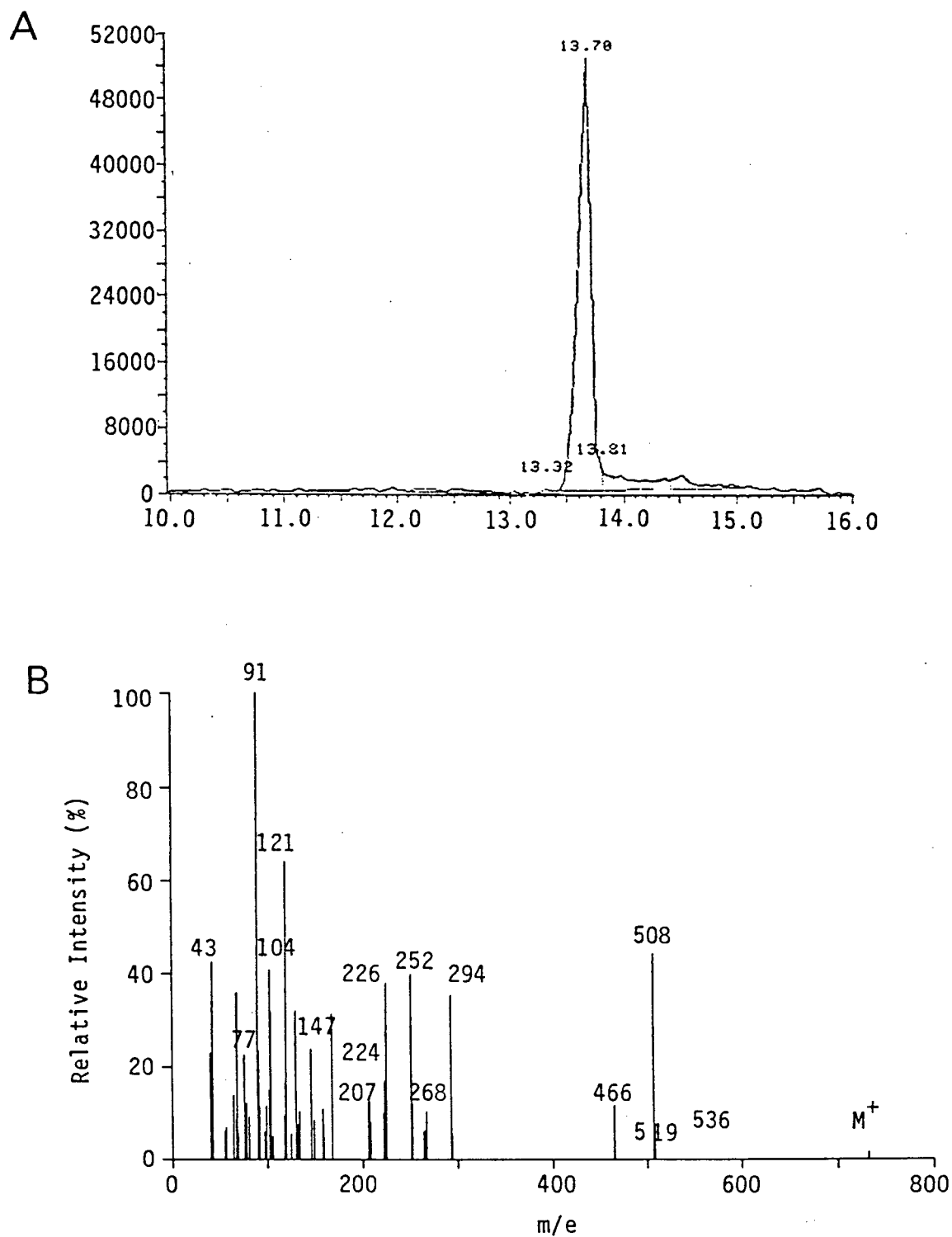


Figure 25. GLC-EI-MS of HFB-PF: (A) Total ion chromatogram (retention time, 13.70 min) and (B) EI mass spectrum.

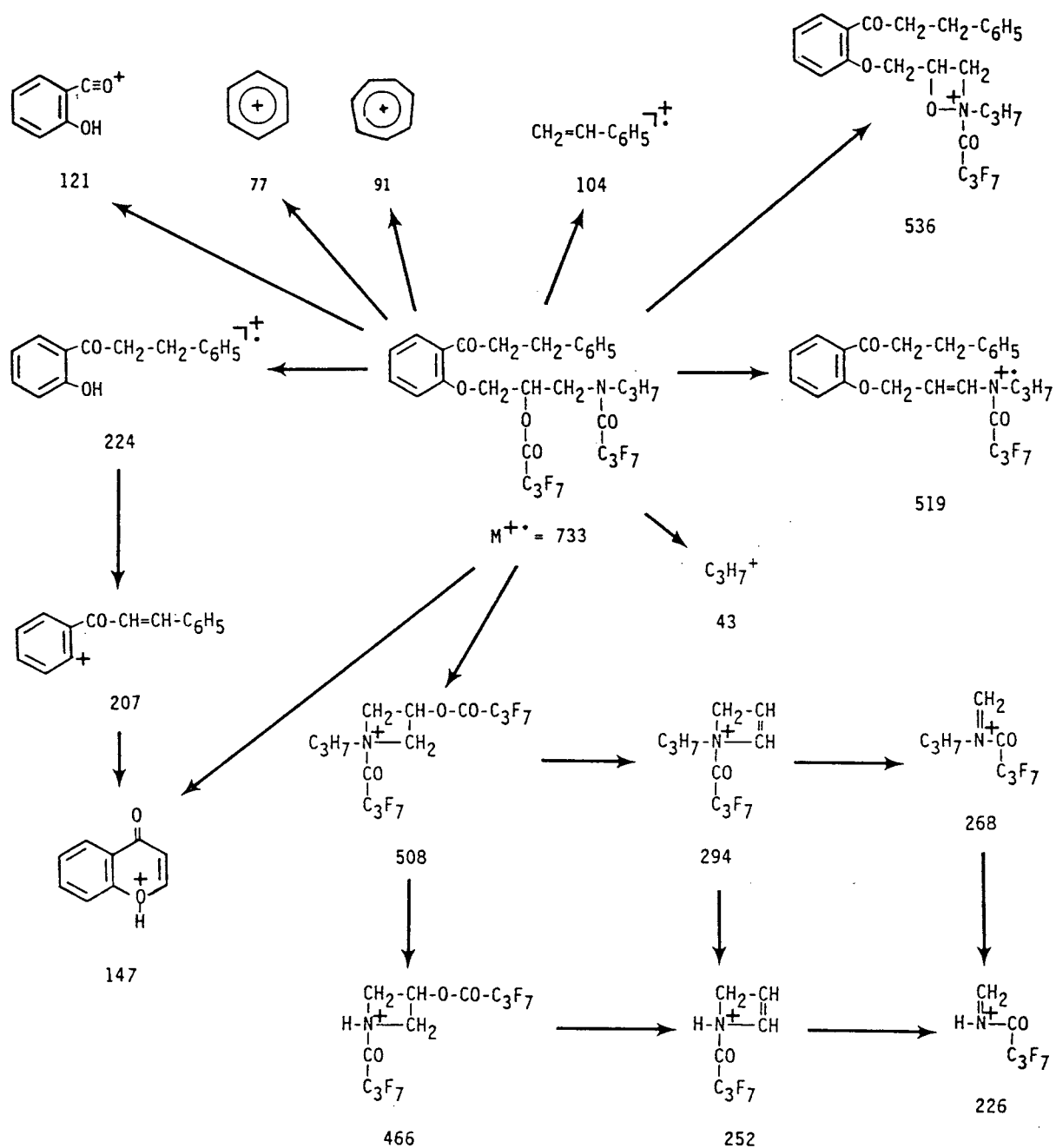


Figure 26. GLC-EI-MS: a proposed fragmentation pattern of HFB-PF.

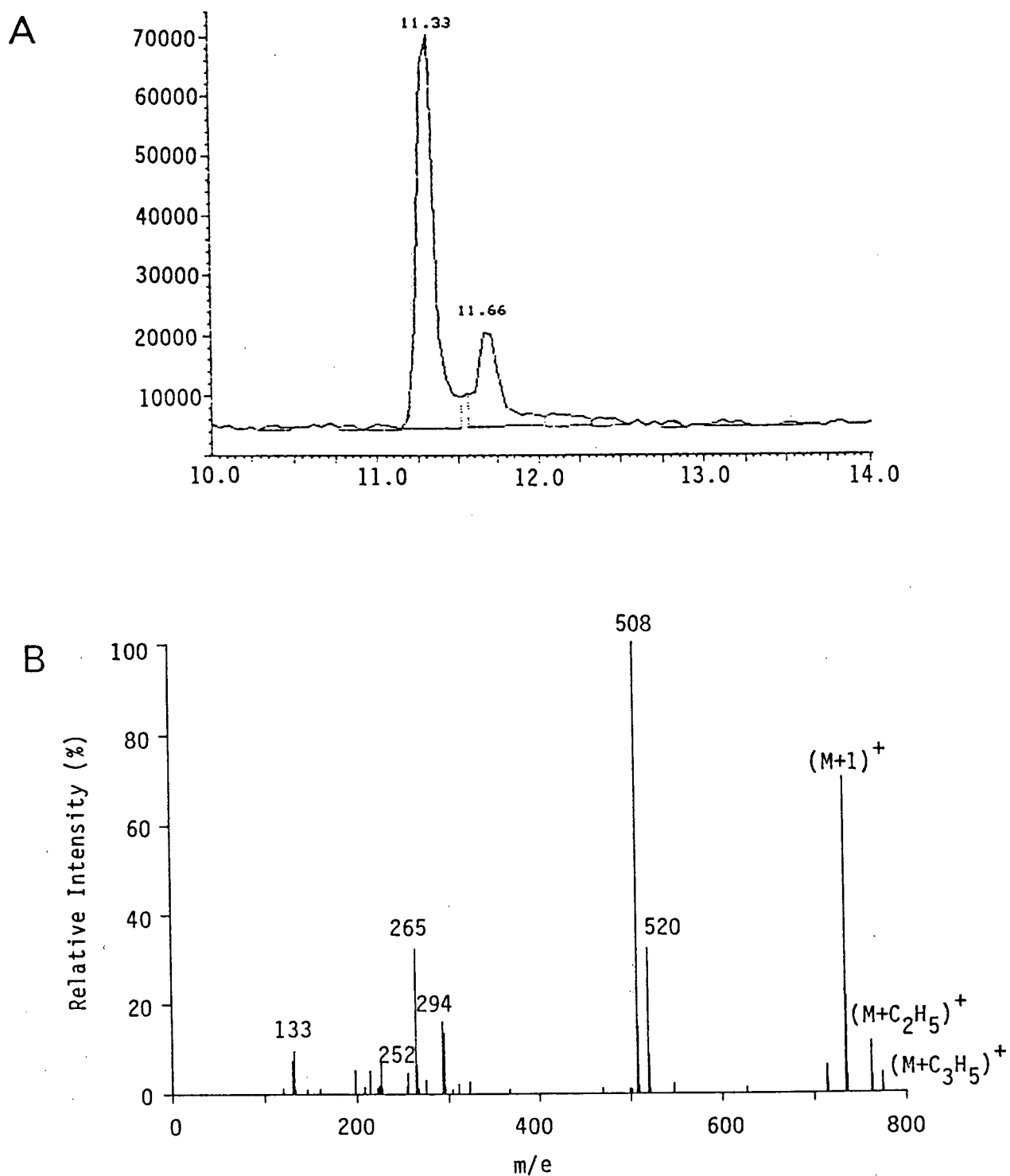


Figure 27. GLC-PICI-MS of HFB-PF: (A) Total ion chromatogram (retention time, 11.33 min) and (B) PICI mass spectrum.

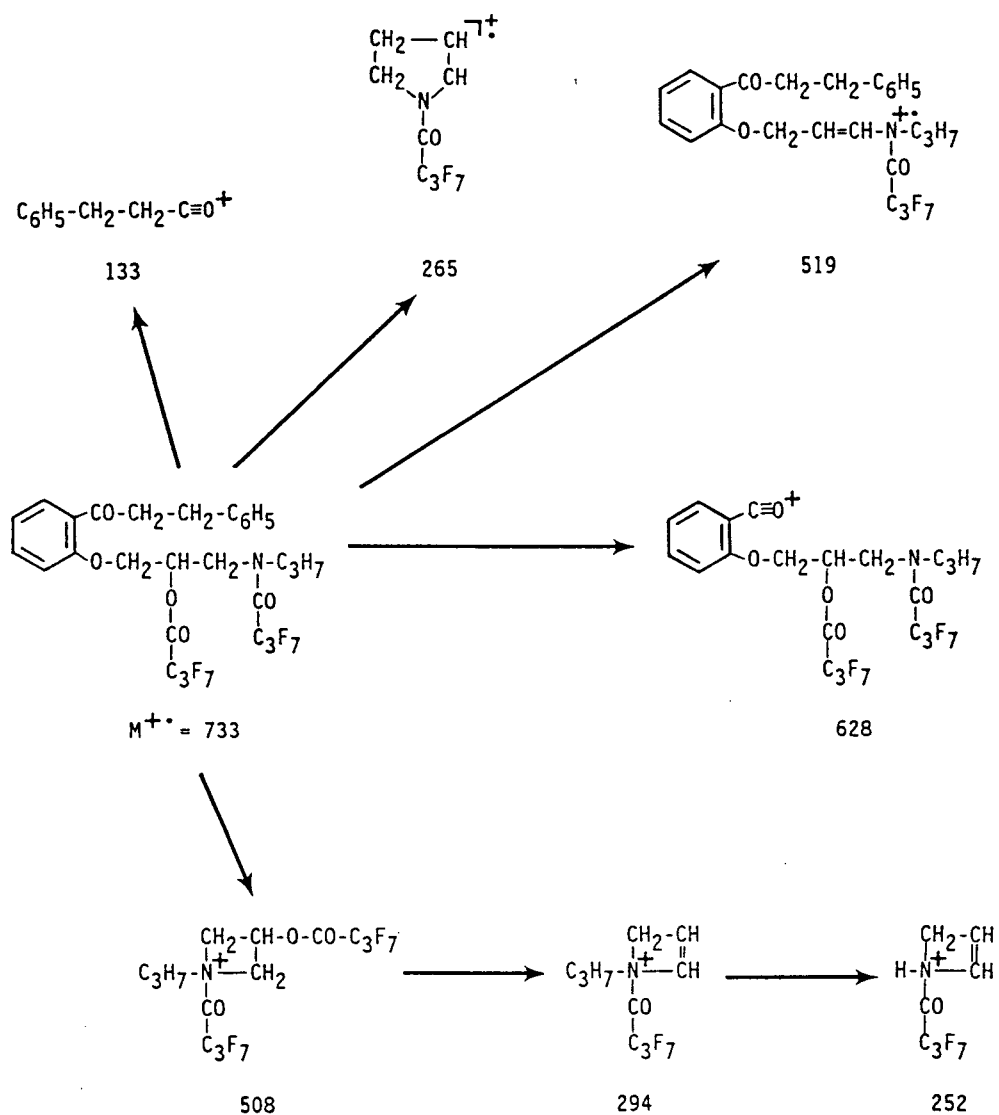


Figure 28. GLC-PICI-MS: a proposed fragmentation pattern of the HFB-PF.

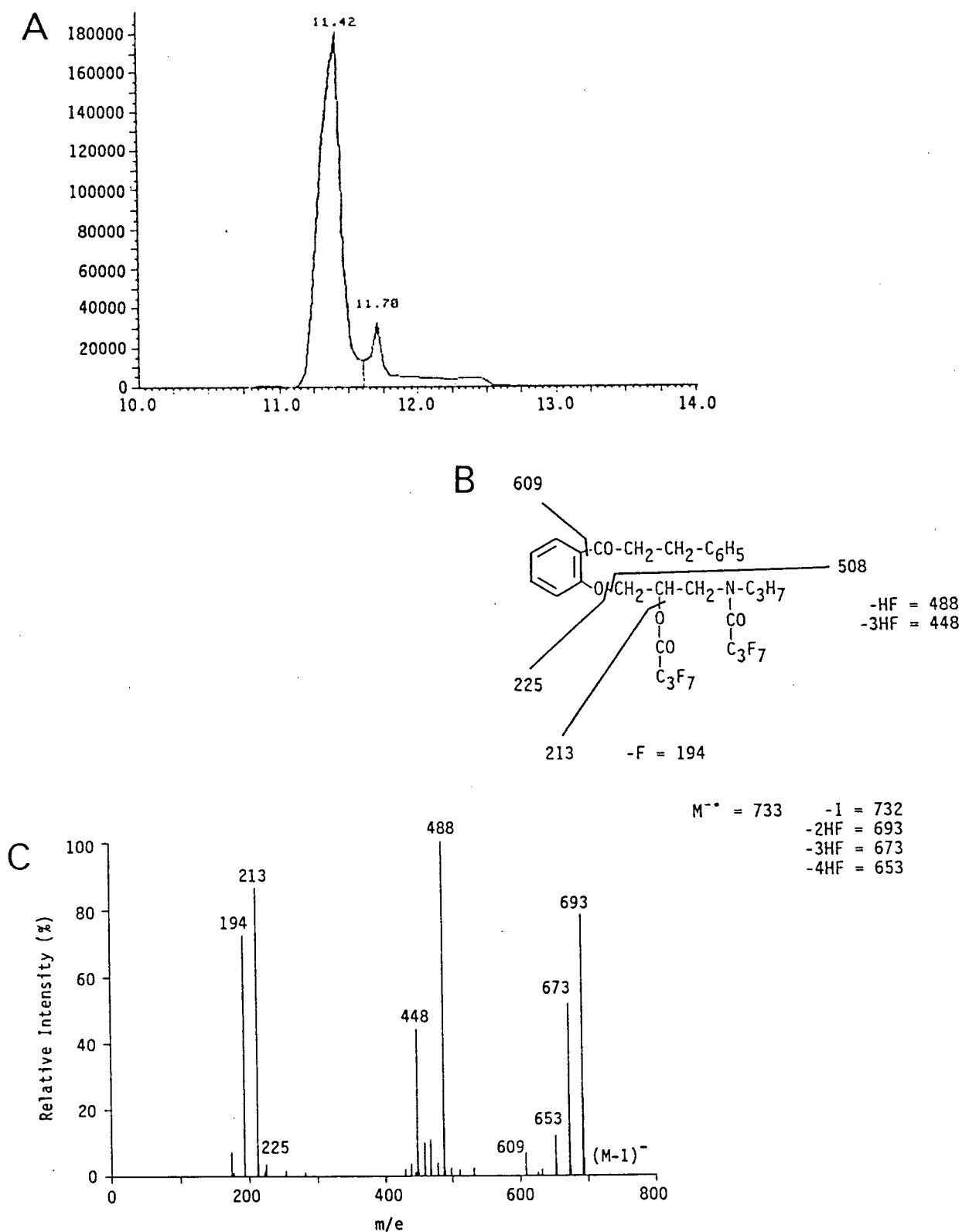
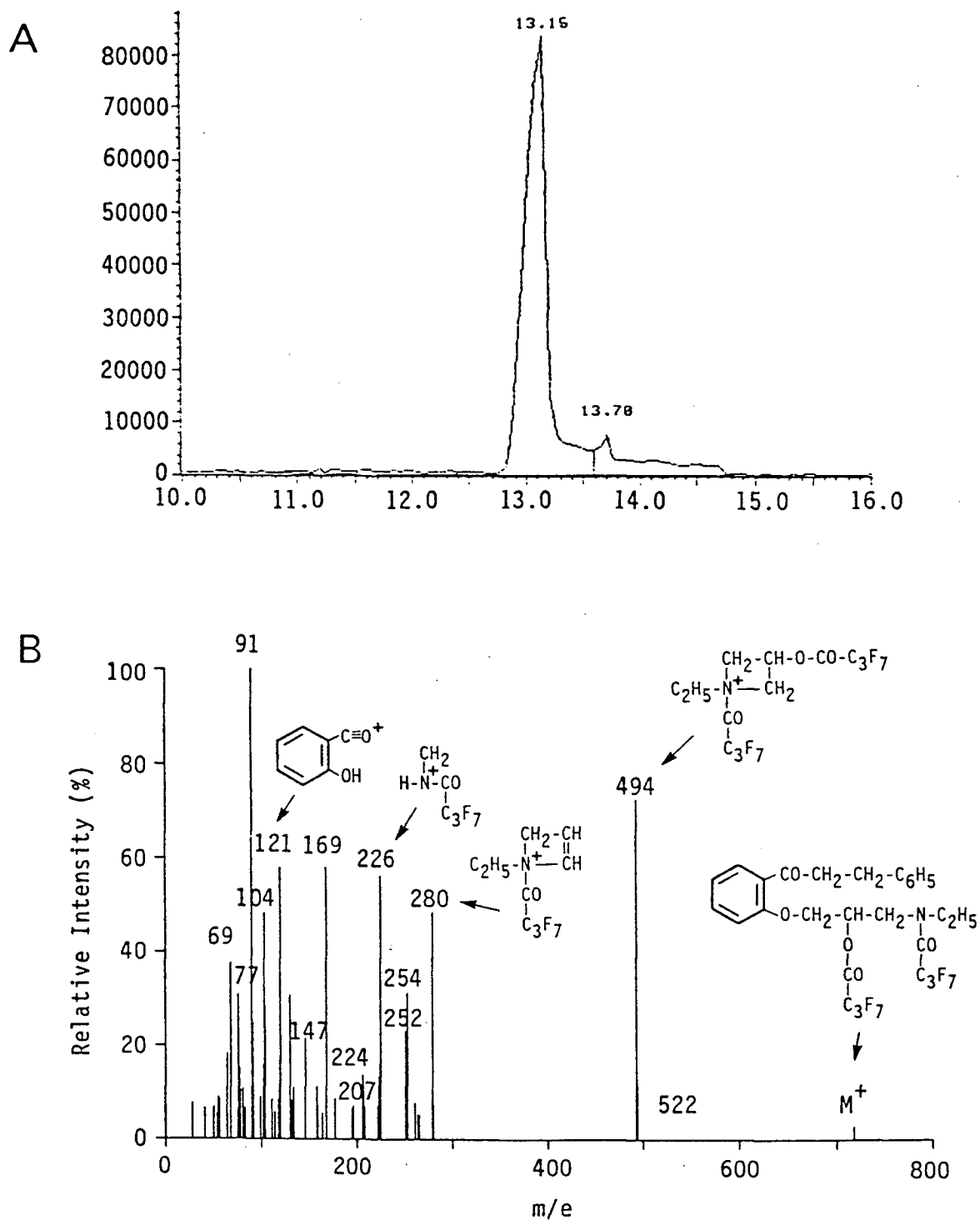


Figure 29. GLC-NICI-MS of HFB-PF: (A) Total ion chromatogram (retention time, 11.42 min); (B) a proposed fragmentation pattern and (C) NICI mass spectrum.



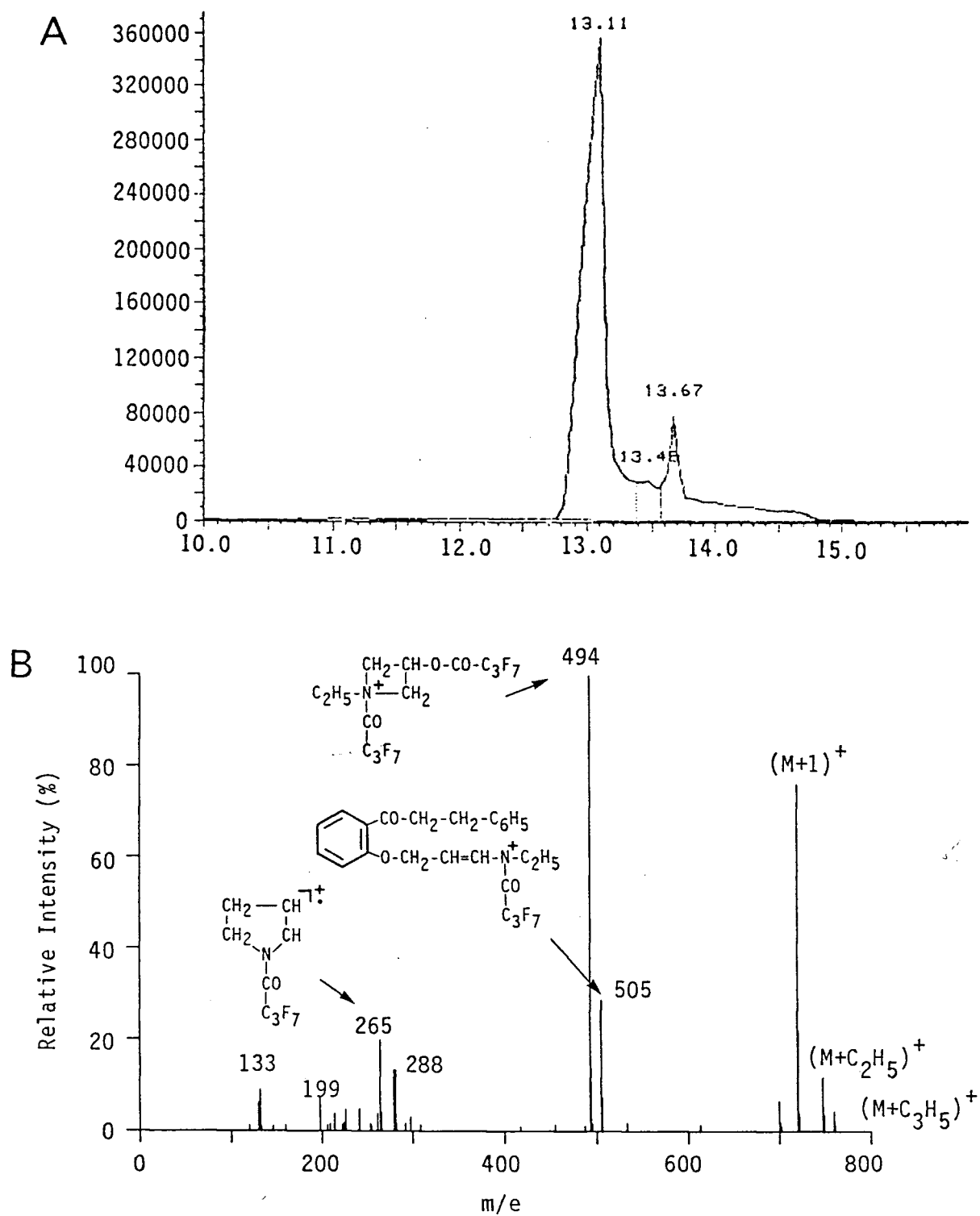
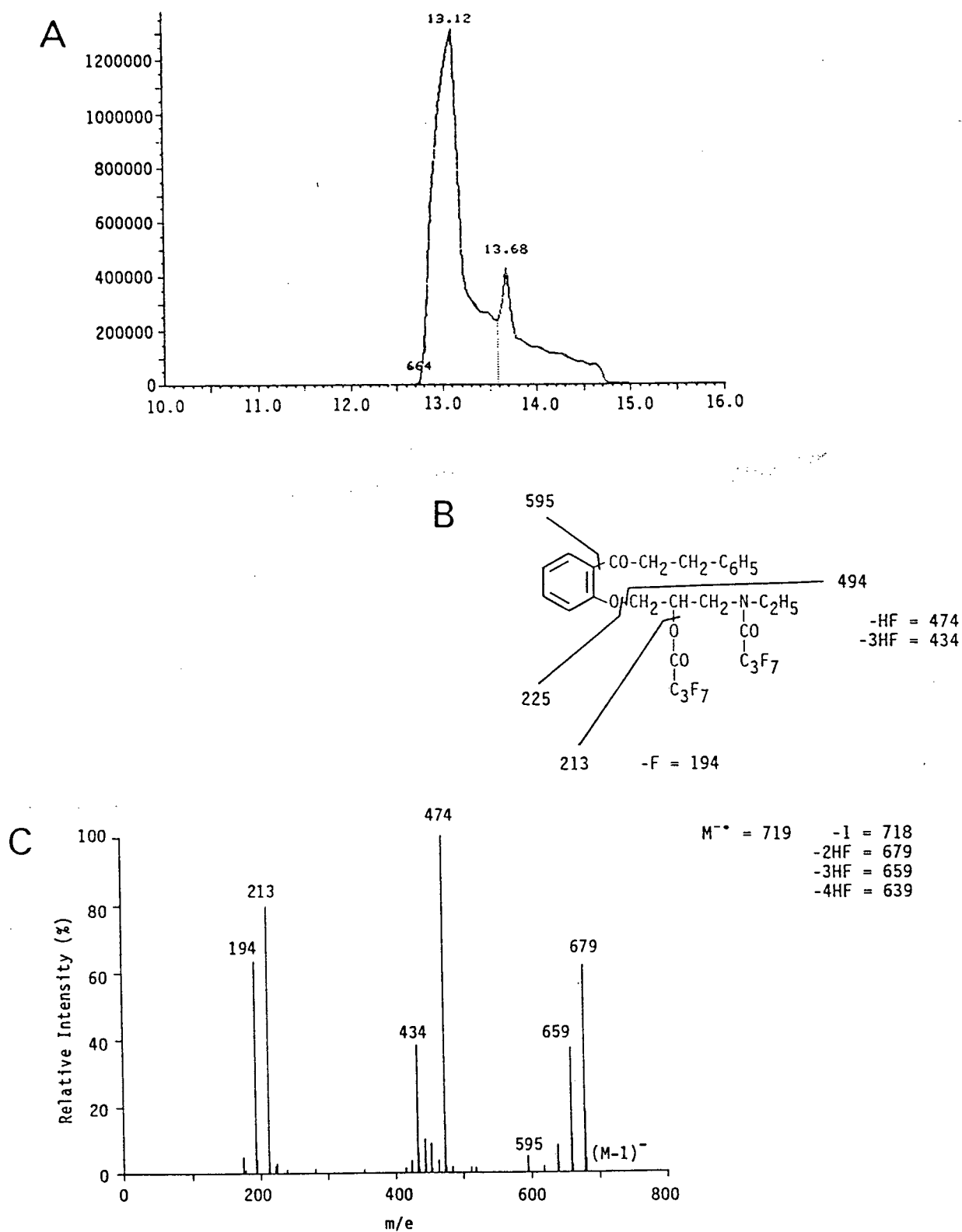


Figure 31. GLC-PICI-MS of HFB-I.S.-a: (A) Total ion chromatogram (retention time, 13.11 min) and (B) PICI mass spectrum and selected proposed fragmentation structures.



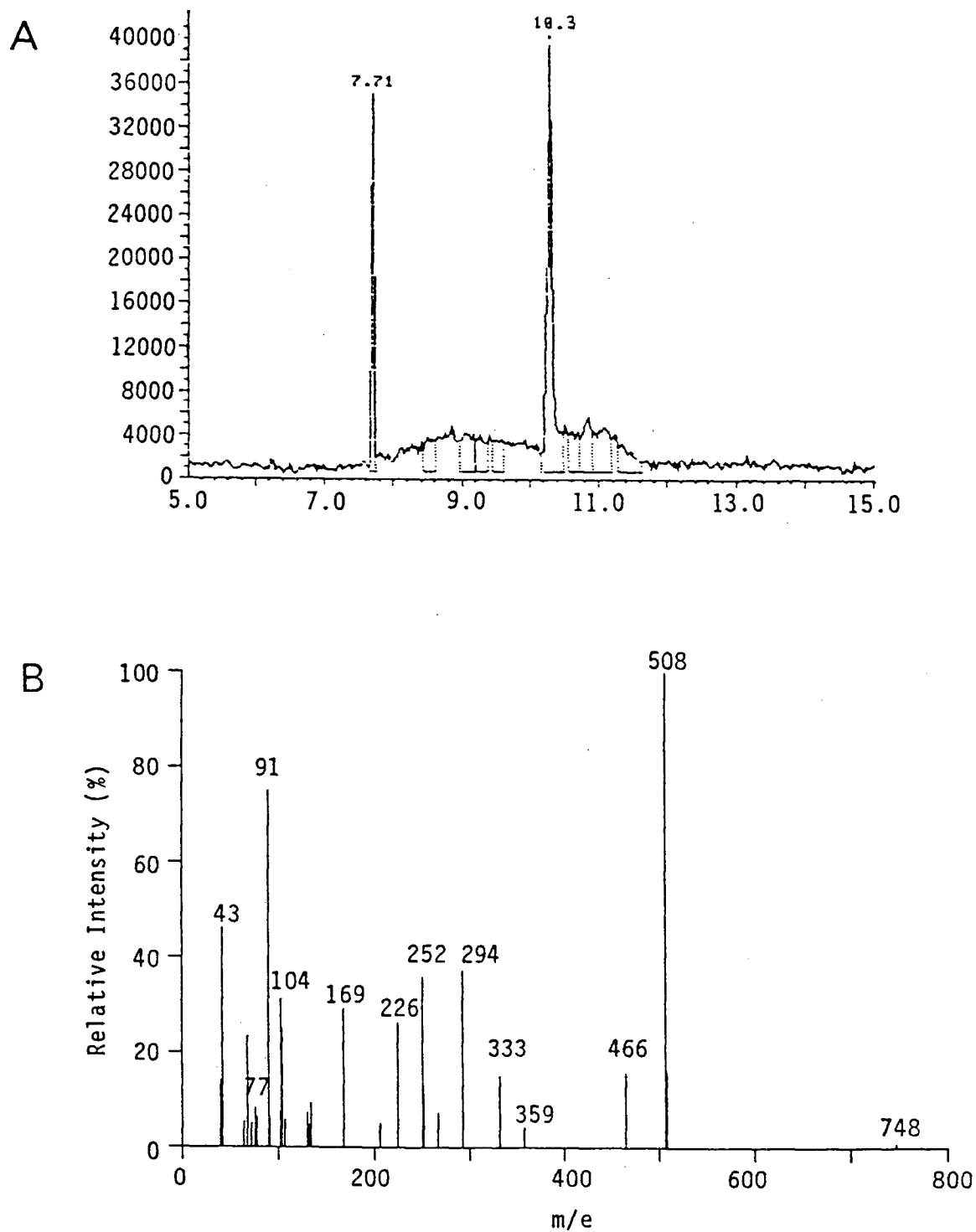


Figure 33. GLC-EI-MS of HFB-5-hydroxy PF: (A) Total ion chromatogram (retention time, 10.3 min) and (B) EI mass spectrum.

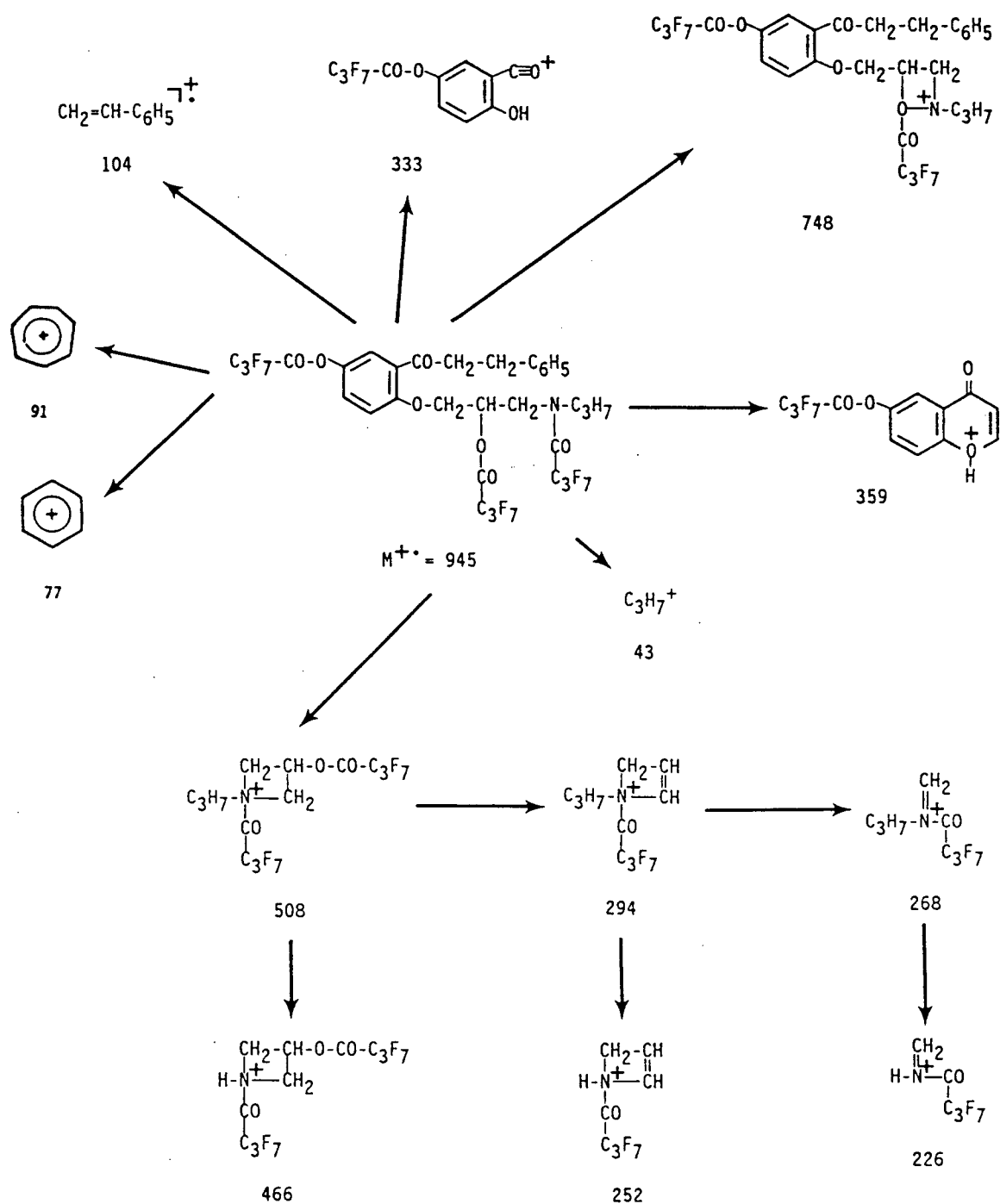


Figure 34. GLC-EI-MS: a proposed fragmentation pattern of HFB-5-hydroxy PF.

chromatogram, fragmentation pattern and MS spectrum of 5-hydroxy PF. In order of decreasing intensity, the prominent fragment ions were at m/e 508, 91, 43, 294, 252, 104, 169, 226, 466, 333, 359, 77, 748. The molecular ion (M^+) at m/e 945 was not observed on the EI-MS spectrum.

3.4 Measurement of Plasma Propafenone Concentrations by Electron-Capture Detection Gas-Liquid Chromatography and High-Performance Liquid Chromatography

Table 10 shows steady-state trough plasma PF concentrations in patients receiving PF for control of cardiac dysrhythmias. Also contained in Table 10 are the results of a comparison of PF plasma concentrations analyzed by our GLC assay and by a modification of the HPLC method reported by Harapat and Kates [1982]. Statistical analysis (paired t-test, level of significance = 0.05) indicates that there is no significant difference between the results obtained using these two independent methods of PF measurement.

3.5 *In Vitro* Serum Protein Binding Study

3.5.1 Equilibrium Time for Propafenone During Equilibrium Dialysis

The results of the equilibration study to establish the time necessary to reach dynamic equilibrium in the dialysis chambers are presented in Figure 35. Equilibrium, which is independent of PF concentration, was reached between 4 and 6 h. Therefore, 6 h was chosen as

Table 10. Steady-state plasma propafenone trough concentration of patients receiving propafenone.

Patient number	Concentration measured (ng/mL) (mean \pm s.d.)	
	GLC ^a	HPLC ^b
1	175.6 \pm 7.7	- *
2	696.7 \pm 6.1	738.2 \pm 53.4
3	737.5 \pm 7.7	820.2 \pm 5.1
4	5.3 \pm 0.4	-
5	190.8 \pm 10.6	-
6	554.3 \pm 84.4	-
7	217.9 \pm 6.7	-
8	131.4 \pm 6.0	-
9	179.4 \pm 6.8	-
10	476.7 \pm 23.5	482.1 \pm 1.5
11	356.4 \pm 1.7	-
12	373.6 \pm 8.7	385.0 \pm 2.3
13	303.3 \pm 2.2	328.7 \pm 10.2
14	444.9 \pm 2.1	365.6 \pm 77.3
15	612.9 \pm 7.2	608.8 \pm 78.1
16	633.8 \pm 17.5	719.9 \pm 12.9
17	487.3 \pm 35.8	604.5 \pm 5.8
18	28.7 \pm 0.4	-
19	119.2 \pm 6.6	-
20	1740.5 \pm 31.9	1873.8 \pm 8.5

s.d. standard deviation

a number of samples, n = 2 (two injections for each sample)

b number of samples, n = 1 (two injections for each sample),
The method is a slight modification of the HPLC technique of Harapat and Kates [1982].

* PF concentration unmeasurable

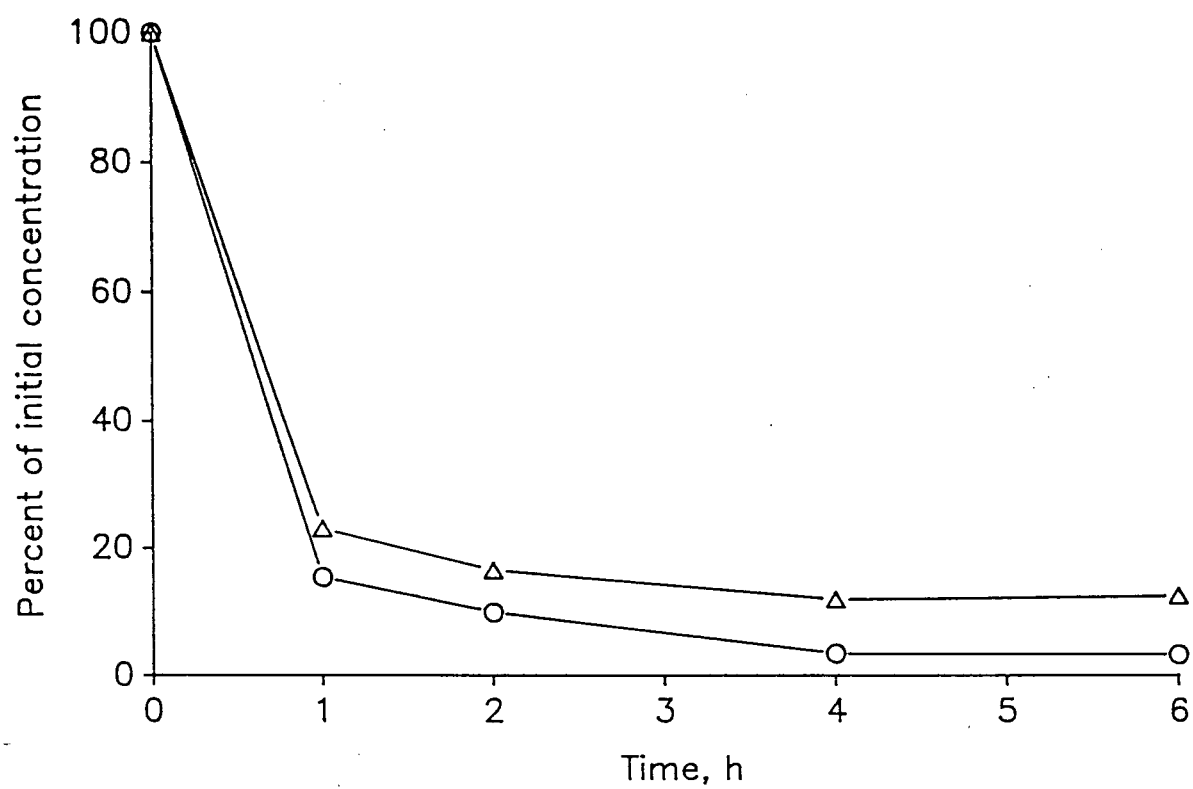


Figure 35. Equilibrium time for equilibrium dialysis of propafenone. Initial propafenone concentration was 0.25 (O) and 100 (Δ) µg/mL.

the optimal time to assure attainment of equilibrium. Theoretically, the approach to equilibrium is faster when dialyzing buffer with serum samples (containing drug already bound to serum proteins). Therefore, 6 h was used as the dialysis time when measuring the binding of PF in serum samples containing PF.

3.5.2 Non-Specific Binding of Propafenone

The extent of the non-specific adsorption of PF to the equilibrium dialysis membrane, equilibrium dialysis cell and ultrafiltration device is shown in Table 11 and the mean percentage of PF adsorbed was $2.3 \pm 1.7 \%$, $16.6 \pm 8.0 \%$ and $16.2 \pm 7.2 \%$, respectively.

3.5.3 Determination of Propafenone Binding Parameters - Rosenthal Analysis

The binding ratio (bound concentration/free concentration) of PF in serum from six healthy subjects was plotted versus the bound concentration of PF by the method of Rosenthal [1967] (Figure 36). Two classes of binding sites were observed over the concentration range studied. The affinity and capacity constants for each binding site are also shown in Figure 36. The mean values for the high-affinity, low-capacity binding site are $K_1 = 6.53 \times 10^5 \text{ M}^{-1}$ and $n_1P_1 = 1.73 \times 10^{-4} \text{ M}$; for the low-affinity, high-capacity site are $K_2 = 8.77 \times 10^3 \text{ M}^{-1}$ and $n_2P_2 = 8.57 \times 10^{-3} \text{ M}$.

3.5.4 Scatchard Plot of the Binding Data

The binding data from the same six healthy subjects, plotted as r' versus the ratio of $r'/\text{free drug concentration}$ (Scatchard plot), is shown in Figure 37A and B. Serum albumin (M.W. ~65,000) (Figure 37A) and AAG

Table 11. Non-specific adsorption of propafenone to the equilibrium dialysis membrane, dialysis cell and ultrafiltration device.

Concentration of propafenone ($\mu\text{g/mL}$)	Percentage of propafenone adsorbed		
	Equilibrium dialysis membrane	Equilibrium dialysis cell	Ultrafiltration device
0.1	5.0	21.6	21.3
0.5	0.8	22.3	25.1
1.0	2.5	23.3	19.7
5.0	3.2	19.5	15.5
10.0	1.7	7.7	8.8
100.0	0.3	5.1	6.9
Mean	2.3	16.6	16.2
\pm s.d.	± 1.7	± 8.0	± 7.2

s.d. standard deviation

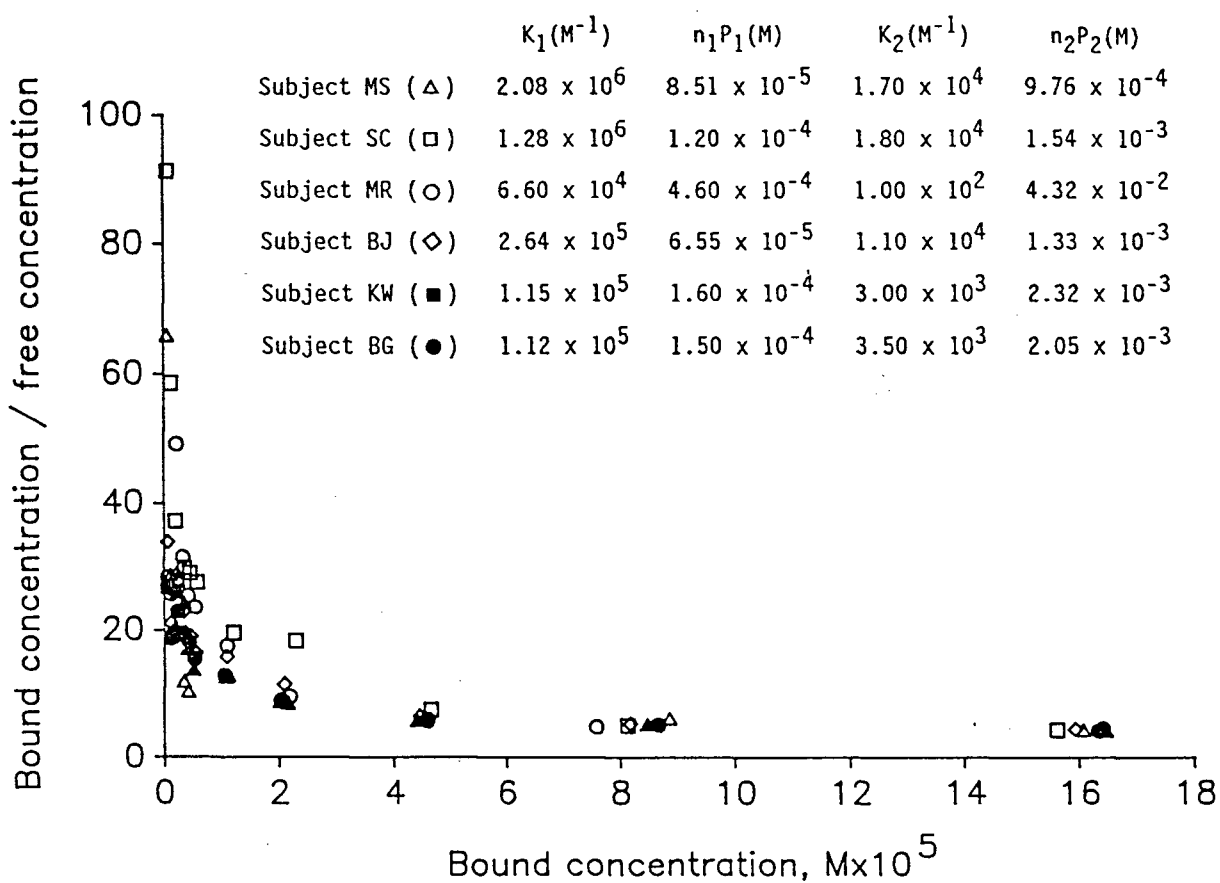


Figure 36. Relationship between the ratio of bound concentration/free concentration and bound concentration of propafenone in the serum of six healthy male subjects (Rosenthal plot).

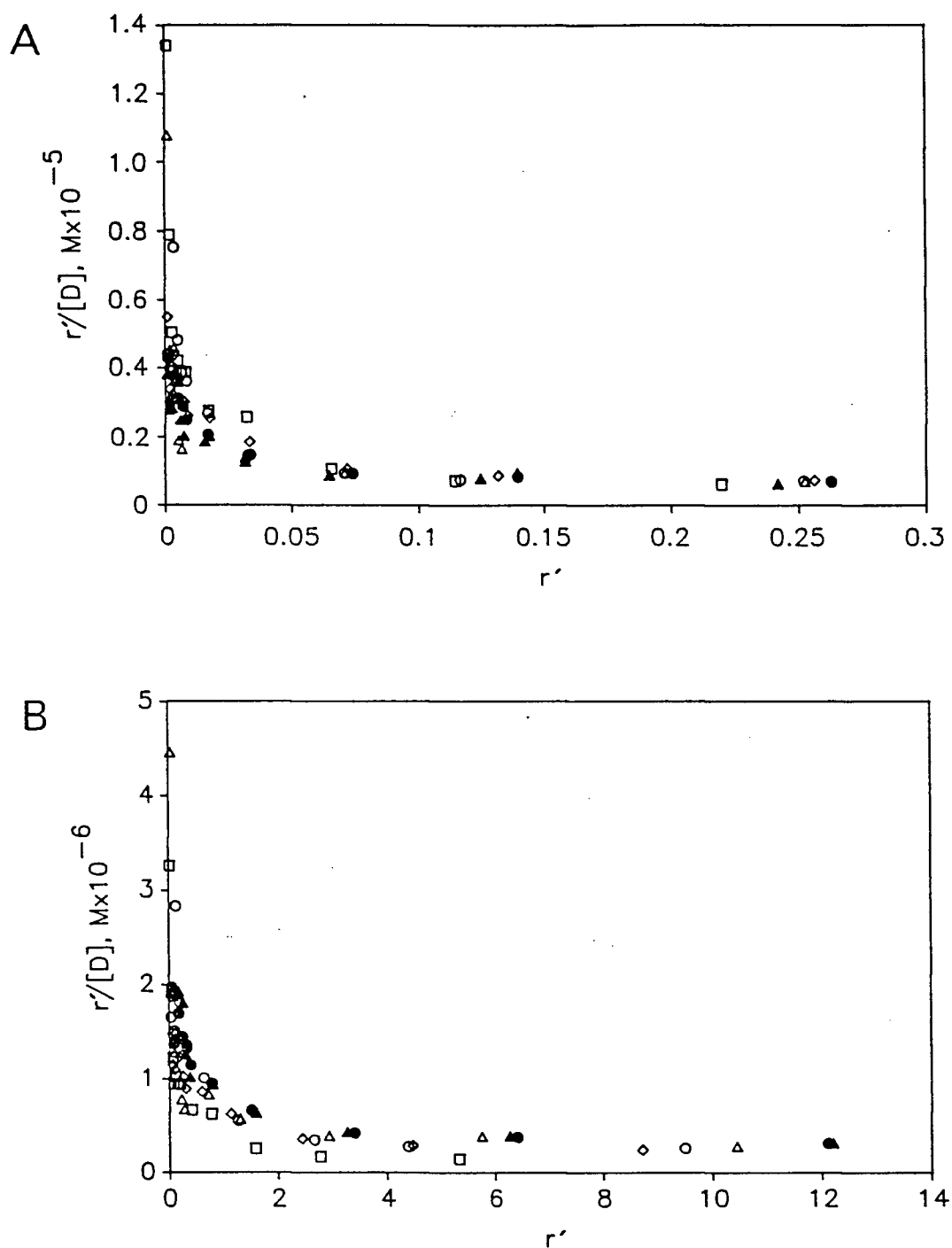


Figure 37. Relationship between $[r'/\text{bound concentration}]$ and $[r']$ of propafenone in the serum of six healthy male subjects (Scatchard plot); serum concentrations of (A) albumin and (B) AAG were used in the calculation of $[r']$.

(M.W. ~40,000) (Figure 37B) concentrations were used in the calculation of r' , respectively. Two classes of binding sites were apparent over the concentration range studied.

3.5.5 Propafenone Free Fraction in Normal and Pooled Uremic Serum

In normal serum, the free fraction of PF was 0.027 ± 0.011 at a PF concentration of $0.25 \mu\text{g/mL}$, 0.041 ± 0.010 within the therapeutic concentration range ($0.5\text{-}2 \mu\text{g/mL}$), 0.138 ± 0.012 at a PF concentration of $25 \mu\text{g/mL}$ and 0.187 ± 0.005 when the PF concentration was increased to $100 \mu\text{g/mL}$ (Table 12). Regression of free fraction over the entire concentration range studied ($0.25\text{-}100 \mu\text{g/mL}$) was found to be non-linear ($p < 0.05$). When the concentration range of 0.25 to $1.5 \mu\text{g/mL}$ was further tested, the correlation between free fraction and concentration was found to be linear but the slope was not significantly different from zero ($p > 0.05$), indicating that PF free fraction is concentration-independent within this range. The free fraction of PF in pooled uremic serum (AAG concentration = 124 mg/dL) was approximately 50% of the serum free fraction seen in normal serum (AAG concentration range = $53\text{-}117 \text{ mg/dL}$) at each PF concentration studied ($1\text{-}5 \mu\text{g/mL}$).

3.5.6 Effect of Uremia and Renal Failure on the Serum Binding of Propafenone

In patients with chronic renal failure, serum AAG concentration ($140 \pm 39 \text{ mg/dL}$) was twice that in healthy subjects ($71 \pm 24 \text{ mg/dL}$) and mid-range uremic patients ($72 \pm 2 \text{ mg/dL}$). The PF free fraction ranged from 0.012 to 0.042 , with a mean value of 0.024 ± 0.012 (corresponding value in healthy subjects, 0.034 ± 0.007 ; mid-range uremic patients, 0.037 ± 0.008).

Table 12. Propafenone free fraction in normal and pooled uremic serum.

Initial concentration of propafenone ($\mu\text{g/mL}$)	Propafenone free fraction (mean \pm s.d.)	
	Normal serum ^{ab}	Pooled uremic serum ^c
0.25	0.027 \pm 0.011	-*
0.50	0.039 \pm 0.013	-
0.75	0.044 \pm 0.009	-
1.0	0.034 \pm 0.007	0.016 \pm 0.001
1.5	0.041 \pm 0.008	0.026 \pm 0.001
2.0	0.049 \pm 0.010	0.029 \pm 0.002
2.5	0.055 \pm 0.014	0.030 \pm 0.001
5.0	0.064 \pm 0.011	0.034 \pm 0.001
10.0	0.089 \pm 0.020	-
25.0	0.138 \pm 0.012	-
50.0	0.161 \pm 0.006	-
100.0	0.187 \pm 0.005	-

s.d. standard deviation

a mean propafenone free fraction in serum from normal healthy volunteers, n = 6, AAG concentration ranged from 53 to 117 mg/dL

b propafenone free fraction is concentration-independent within the concentration range of 0.25-1.5 $\mu\text{g/mL}$, tested by 'regression with replication' on the linearity (linear, $p < 0.05$) and slope (slope not significantly different from 0, $p > 0.05$)

c propafenone free fraction in pooled serum from nine uremic patients, AAG concentration is 124 mg/dL

* propafenone free fraction not measured

3.5.7 Correlation Between Serum α_1 -Acid Glycoprotein Concentration and Propafenone Binding Ratio

The relationship between serum AAG concentration and the PF binding ratio is shown in Figure 38. There is a positive and significant correlation ($r = 0.830$, $n = 14$, $p < 0.05$) between these two parameters. The best-fit line by linear regression analysis is described by $Y = 0.351X + 3.347$.

3.6. Phenobarbital Treatment in Healthy Non-Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

3.6.1. Serum Data of Propafenone

The semi-logarithmic plots of the serum concentration *versus* time curves of PF from the eight healthy non-smoking subjects are shown in Appendix 2.

The kinetic data of PF in serum before and after phenobarbital treatment is shown in Table 13. All non-smoking subjects were 'fast' metabolizers ($CL_{int} > 0.5$ L/min). Propafenone is a compound with a high intrinsic clearance which exhibits substantial interindividual variability in this parameter. In our non-smoking subjects, CL_{int} ranged from 0.8 to 13.3 L/min in the control state and 1.8 to 69.8 L/min after phenobarbital treatment. There was also a large interindividual variability in other kinetic parameters. For example, a 7 fold difference in C_{max} (range 103-750 ng/mL) and a 16 fold difference in AUC (range 340.5-5465.4 h.ng/mL) were noted between subjects in the control state while a 30 fold difference in C_{max} (range 16-433 ng/mL) and a 39 fold difference in AUC (range 64.7-

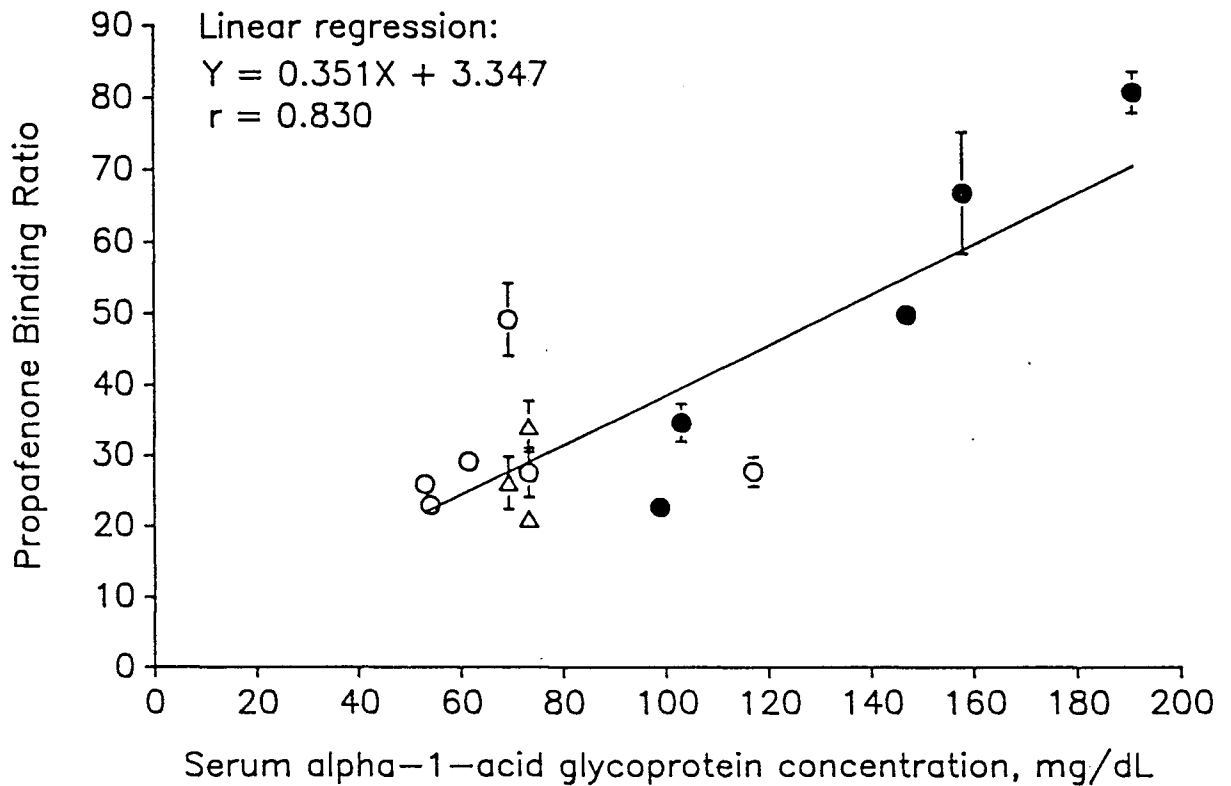


Figure 38. Relationship between propafenone binding ratio (bound concentration/free concentration) and AAG concentration in serum obtained from six healthy subjects (○), three mid-range uremic patients (△) and five patients with chronic renal failure (●). The data are presented as mean ± 1s.d.

Table 13. The kinetic data of propafenone in serum before and after phenobarbital treatment from eight healthy non-smoking subjects.

Subject		$t_{1/2\beta}$ (h)	C_{\max} (ng/mL)	t_{\max} (h)	CL_{int} (L/min)	AUC (h.ng/mL)	% increase in CL_{int}	% decrease in C_{\max}	% decrease in AUC
BK	C	3.4	194	4	3.7	1227.4	62	28	38
	P	3.7	140	5	6.0	756.4			
NP	C	2.5	217	1.5	4.9	923.7	176	58	64
	P	1.9	92	1.5	13.5	333.7			
CA	C	1.4	177	3	6.2	725.6	176	63	64
	P	1.4	66	4	17.1	264.1			
DA	C	2.1	124	3	7.5	602.7	831	87	89
	P	2.3	16	3	69.8	64.7			
SG	C	4.5	129	2	8.3	542.8	10	26	9
	P	2.8	96	3	9.1	496.5			
MV	C	4.4	750	3	0.8	5465.4	125	42	54
	P	4.7	433	3	1.8	2499.5			
GP	C	1.6	328	2	4.3	1052.4	153	53	61
	P	1.7	153	2	10.9	414.0			
UH	C	2.6	103	1.5	13.3	340.5	22	0	18
	P	1.3	103	1.5	16.2	278.2			
Mean ±s.d.	C	2.8 ±1.2	253 ±221	2.5 ±0.9	6.1 ±3.7	1360.1 ±1683.5	194 ±266	45 ±27	50 ±26
	P	2.5 ±1.2	137 ^S ±127	2.9 ±1.2	18.1 ^S ±21.5	638.4 ^S ±778.3			

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

s.d. standard deviation

2499.5 h.ng/mL) were observed after phenobarbital treatment.

Phenobarbital treatment enhanced the first-pass metabolism of PF, as shown by a significant increase in CL_{int} and a significant decrease in C_{max} and AUC. The absolute increase in CL_{int} within the individual subject ranged from 10 to 831%, with a mean of 194%. The absolute decrease in AUC ranged from 9 to 89%, with a mean of 50% while the decrease in C_{max} ranged from 0 to 87%, with a mean of 45%. In addition, the percent decrease in C_{max} was not significantly different from the percent decrease in AUC.

The $t_{1/2\beta}$ of PF ranged from 1.4 to 4.5 h (mean 2.8 h) in the control state. No significant change in the $t_{1/2\beta}$ was observed after phenobarbital treatment (range 1.3-4.7 h, mean 2.5 h)

The t_{max} of PF ranged from 1.5 to 4 h (mean 2.5 h) in the control state. Phenobarbital treatment did not affect the t_{max} (range 1.5-5 h, mean 2.9 h).

Table 14 shows the values of V_{darea}/F and V_{dss}/F of the non-smokers before and after phenobarbital treatment. In the control state, V_{darea}/F ranged from 3 to 38.4 L/kg and V_{dss}/F ranged from 3.5 to 45.7 L/kg. Except for subjects SG and UH, there was a 2 to 10 fold increase in both V_{darea}/F and V_{dss}/F after phenobarbital treatment. The increase in V_{dss}/F was significant while the increase in V_{darea}/F was not. The values of V_{dss}/F were, in general, higher than V_{darea}/F , the difference being statistically significant.

3.6.2. Salivary Data of Propafenone

The salivary concentration *versus* time curves of PF from the eight non-smoking subjects are shown in Appendix 3. Most subjects showed significant fluctuations in salivary PF concentrations over time. However,

Table 14. The volume of distribution of propafenone before and after phenobarbital treatment from eight healthy non-smoking subjects.

Subject		V_{darea}/F (L/kg)*	V_{dss}/F (L/kg)*
BK	C	10.7	15.8
	P	19.1	30.0
NP	C	12.9	16.8
	P	31.5	38.5
CA	C	9.3	14.0
	P	25.6	52.6
DA	C	18.5	22.2
	P	190.0	257.7
SG	C	30.6	32.9
	P	21.0	30.7
MV	C	3.0	3.5
	P	7.5	7.6
GP	C	5.9	8.8
	P	15.1	26.5
UH	C	38.4	45.7
	P	28.6	42.2
Mean \pm s.d.	C	16.2 \pm 12.4	20.0 \pm 13.6
	P	42.3 \pm 60.2	60.7 \pm 80.7 ^S

C control (untreated)

P phenobarbital-treated

* normalized to the individual's body weight

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

s.d. standard deviation

the reduction in salivary PF concentrations caused by phenobarbital mirrored the changes noted in serum PF concentrations.

The kinetic data of PF in saliva before and after phenobarbital treatment is shown in Table 15. The saliva/serum PF concentration ratios were 0.21 ± 0.08 in the control state and 0.27 ± 0.11 after phenobarbital treatment, with large inter- and intraindividual variation. In two subjects, the saliva/serum PF concentration ratio ranged from 0.03 to 0.17 (subject MV, control) and 0.05 to 1.19 (subject UH, phenobarbital-treated). The saliva/serum PF concentration ratios were inversely correlated with the pH of saliva both in the control state ($r = -0.461$, $n = 72$, $p < 0.05$) and after phenobarbital treatment ($r = -0.422$, $n = 53$, $p < 0.05$). The best fit through the data points was $Y = 1.916 - 0.244X$ (control) and $Y = 2.473 - 0.323X$ (phenobarbital-treated). The salivary AUC_0^t of PF in the control state ranged from 70.7 to 391.7 h.ng/mL. There was a significant decrease in AUC_0^t after phenobarbital treatment. The absolute reduction in AUC_0^t after phenobarbital treatment ranged from 1 to 82%, with a mean of 44%.

3.6.3. Serum Data of 5-Hydroxy Propafenone

The serum concentration *versus* time curves of 5-hydroxy PF from the eight non-smoking subjects are shown in Appendix 4. Serum concentrations of 5-hydroxy PF were lower than the serum concentrations of PF.

The kinetic data of 5-hydroxy PF in serum is shown in Table 16. There was also a large interindividual variation in the kinetic parameters of the metabolite. For example, a 3 fold difference in C_{max} (range 80-245 ng/mL) and AUC (range 412.2-1359.2 h.ng/mL) between subjects was observed in the control state while a 9 fold difference in C_{max} (range 19-177 ng/mL) and an 11 fold difference in AUC (range 78.3-878.0 h.ng/mL) was noted after

Table 15. The kinetic data of propafenone in saliva before and after phenobarbital treatment from eight healthy non-smoking subjects.

Subject		saliva/serum PF concentration ratio	AUC_0^t (h.ng/mL)	% decrease in AUC_0^t
BK	C	0.29 \pm 0.09	319.1	54
	P	0.23 \pm 0.09	147.5	
NP	C	0.14 \pm 0.07	130.1	55
	P	0.21 \pm 0.13	58.8	
CA	C	0.28 \pm 0.15	170.9	66
	P	0.25 \pm 0.12	58.7	
DA	C	0.30 \pm 0.15	127.4	82
	P	0.44 \pm 0.18	23.5	
SG	C	0.22 \pm 0.13	178.8	1
	P	0.39 \pm 0.32	177.7	
MV	C	0.09 \pm 0.05	391.7	44
	P	0.10 \pm 0.05	220.0	
GP	C	0.14 \pm 0.09	133.2	44
	P	0.22 \pm 0.14	74.8	
UH	C	0.25 \pm 0.16	70.7	9
	P	0.33 \pm 0.38	64.2	
Mean \pm s.d.	C	0.21 \pm 0.08	190.2 \pm 108.8	44 \pm 27
	P	0.27 \pm 0.11	103.2 ^S \pm 69.5	

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

s.d. standard deviation

Table 16. The kinetic data of 5-hydroxy propafenone in serum before and after phenobarbital treatment from eight healthy non-smoking subjects.

Subject		$t_{1/2\beta}$ (h)	C_{\max} (ng/mL)	t_{\max} (h)	AUC (h.ng/mL)	% decrease in C_{\max}	% decrease in AUC
BK	C	3.1	118	5	826.5	36	49
	P	2.4	75	5	425.1		
NP	C	3.4	130	2	449.3	28	37
	P	3.2	94	1.5	285.2		
CA	C	1.7	105	3	482.8	17	24
	P	2.3	87	3	366.9		
DA	C	3.8	80	1.5	412.2	76	81
	P	1.9	19	3	78.3		
SG	C	4.1	91	2	413.4	59	35
	P	4.8	37	3	267.0		
MV	C	4.8	126	5	1359.2	20	35
	P	4.3	101	3	878.0		
GP	C	2.0	245	2	756.6	60	61
	P	2.6	98	2	294.8		
UH	C	1.9	231	1.5	780.8	23	36
	P	1.9	177	1.5	501.8		
Mean ±s.d.	C	3.1 ±1.1	141 ±62	2.8 ±1.5	685.1 ±323.7	40 ±22	45 ±18
	P	2.9 ±1.1	86 ^S ±47	2.8 ±1.1	387.1 ^S ±234.4		

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

s.d. standard deviation

phenobarbital treatment. There was a significant decrease in both C_{\max} and AUC after phenobarbital treatment. The absolute decrease in C_{\max} ranged from 17 to 76%, with a mean of 40%, while the decrease in AUC ranged from 24 to 81%, with a mean of 45%.

The $t_{1/2\beta}$ ranged from 1.7 to 4.8 h (mean 3.1 h) in the control state. No significant change in the $t_{1/2\beta}$ was observed after phenobarbital treatment (range 1.9-4.8 h, mean 2.9 h).

The t_{\max} of 5-hydroxy PF ranged from 1.5 to 5 h (mean 2.8 h) in the control state. Phenobarbital treatment did not change the t_{\max} (range 1.5-5 h, mean 2.8 h).

3.6.4. Protein Binding and Serum α_1 -Acid Glycoprotein Concentration

The free fraction of PF (2 and 4 h samples) was 0.027 ± 0.011 in the control state and 0.038 ± 0.032 after phenobarbital treatment. Serum AAG concentrations (0 h samples) in the non-smokers ranged from 69.3 to 108.0 mg/dL (mean 81.1 mg/dL) in the control state and 61.5 to 103.0 mg/dL (mean 77.7 mg/dL) after phenobarbital treatment. Twenty-three days of phenobarbital treatment did not cause any significant change in either PF free fraction (Figure 39) or serum AAG concentration (Figure 40).

Table 17 shows the saliva/serum PF concentration ratios and salivary pH of the 2 and 4 h samples and allows comparison of the values of percent of free (unbound) PF in these samples obtained by two different methods. The percent of free PF in serum, calculated from the data of PF serum/saliva concentration ratio, pH of saliva and pK_a of PF using Equation 7, was significantly higher than the values obtained from equilibrium dialysis.

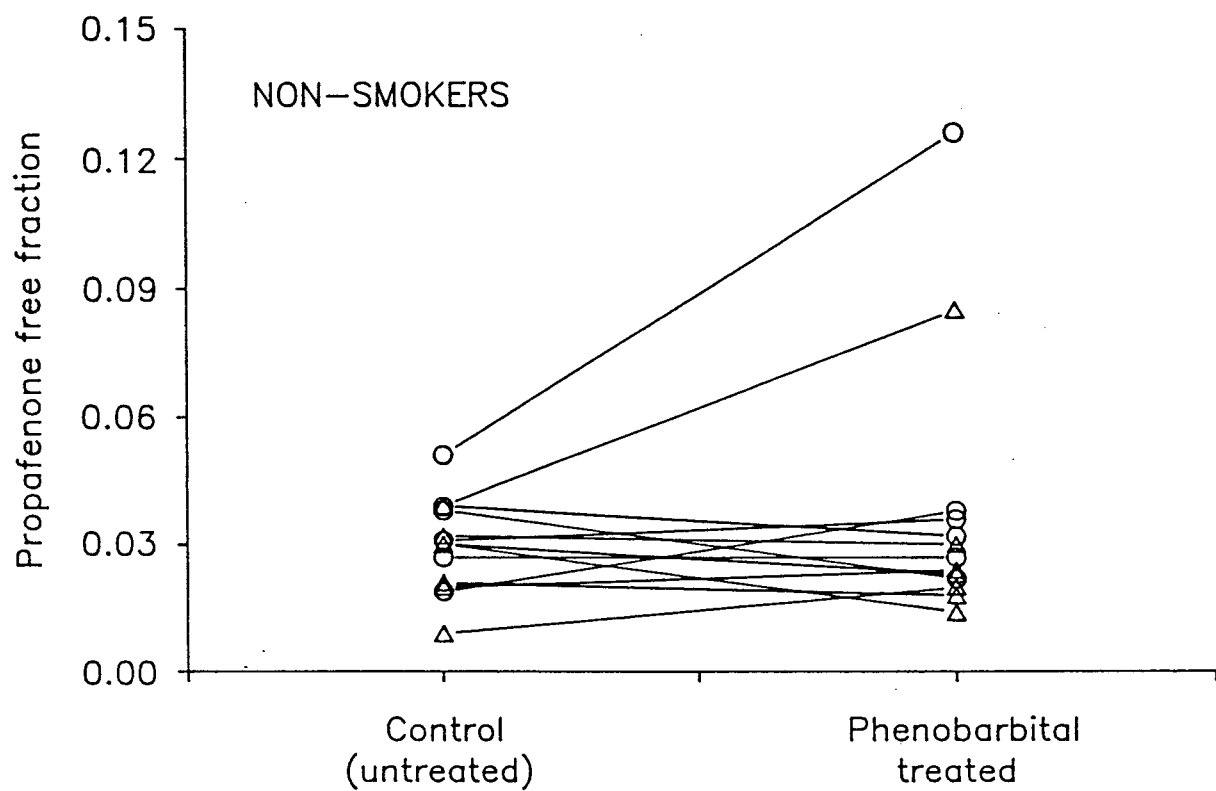


Figure 39. The free fraction of propafenone [2 (O) and 4 (Δ) h samples] before and after 23 days of phenobarbital treatment in eight healthy non-smokers.

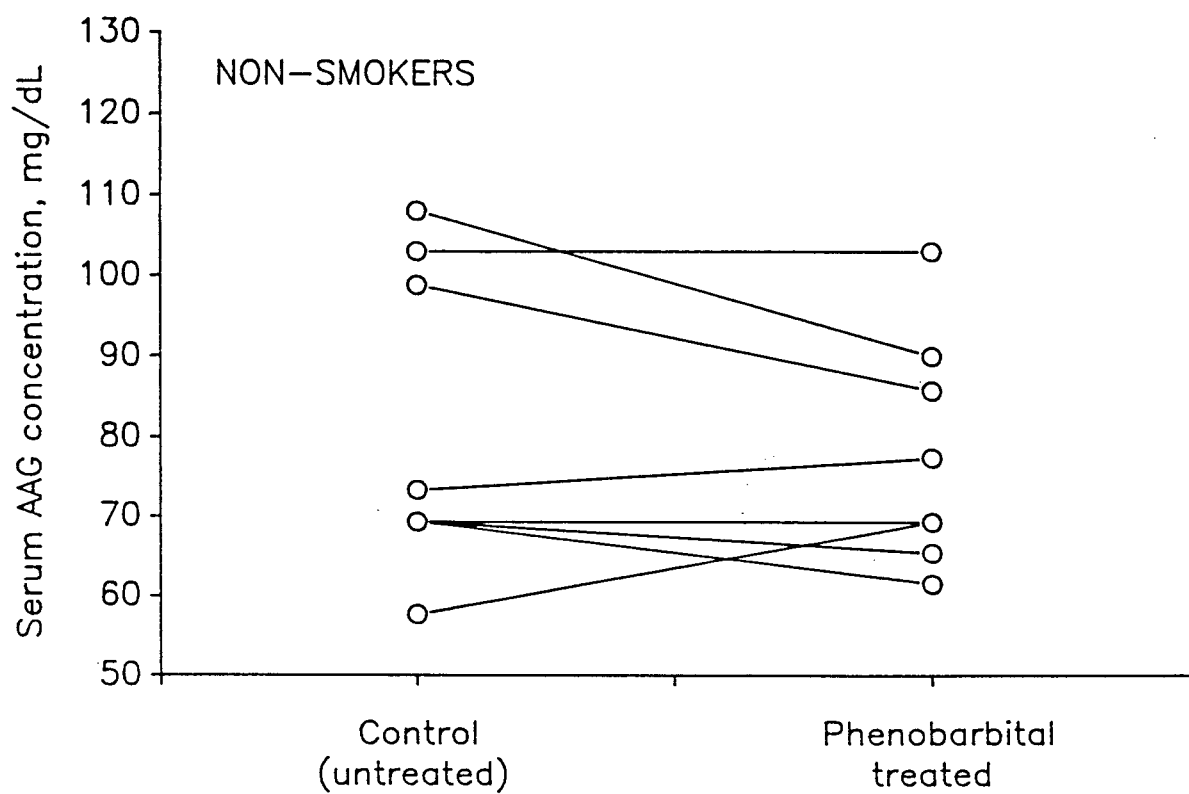


Figure 40. The serum AAG concentration (0 h sample) before and after 23 days of phenobarbital treatment in eight healthy non-smokers.

Table 17. The saliva/serum concentration ratio of propafenone, pH of saliva and percent of free PF from eight healthy non-smoking subjects.

Subject		Sampling time (h)	Propafenone concentration ratio (saliva/serum)	pH of saliva	Serum protein binding (% free)	
					observed ^a	calculated ^b
BK	C	2	0.22	6.80	3.8	5.7
		4	0.30	6.95	3.0	10.9
	P	2	0.17	7.00	2.2	7.0
		4	0.27	6.93	1.4	9.1
NP	C	2	0.12	7.15	1.9	6.8
		4	0.22	7.00	3.0	8.9
	P	2	0.14	7.00	3.8	5.7
		4	0.26	6.92	2.3	8.8
CA	C	2	0.20	7.00	1.1	8.1
		4	0.20	7.01	0.9	8.3
	P	2	—*	7.07	—	—
		4	0.21	6.97	2.0	7.9
DA	C	2	0.28	6.70	2.0	5.7
		4	0.23	6.82	2.1	6.2
	P	2	0.28	6.63	—	4.9
		4	0.33	6.55	—	4.8
SG	C	2	0.16	7.05	2.7	7.2
		4	0.15	6.92	2.0	5.0
	P	2	0.10	7.08	2.7	4.8
		4	0.48	6.85	2.4	13.8
MV	C	2	0.07	7.32	5.1	5.8
		4	0.08	7.22	3.9	5.3
	P	2	0.08	7.05	12.6	3.6
		4	0.08	7.50	8.5	10.0
GP	C	2	0.14	6.94	3.1	4.9
		4	0.12	7.35	2.1	10.7
	P	2	0.08	6.97	3.6	3.0
		4	0.27	6.75	1.8	6.2
UH	C	2	0.12	6.87	3.9	3.6
		4	0.38	6.83	3.2	10.4
	P	2	0.14	6.79	3.2	3.5
		4	0.45	6.74	3.0	10.0

C control (untreated)

P phenobarbital-treated

a obtained from equilibrium dialysis, n=3

b calculated using Equation 7

Wilcoxon paired-sample test, compared to the observed values, significant, $p < 0.05$

* values unavailable

3.6.5. Relationship Between Serum Total, Serum Free and Salivary Propafenone Concentrations

Figure 41 shows the relationship between PF serum total concentration, serum free concentration and salivary concentration of the 2 and 4 h samples (control and phenobarbital-treated) in the non-smoking subjects. The linear regression and correlations are described as follows: PF serum total concentration and PF salivary concentration, $Y = 0.060X + 13.656$, $r = 0.702$ ($n = 31$, $p < 0.05$); PF serum total concentration and PF serum free concentration, $Y = 0.055X - 2.309$, $r = 0.838$ ($n = 29$, $p < 0.05$); PF salivary concentration and PF serum free concentration, $Y = 0.353X - 1.525$, $r = 0.435$ ($n = 29$, $p < 0.05$).

3.6.6. Urinary Data

The data for a representative calibration curve used in the quantitation of the glucuronide and sulfate conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF in urine samples are presented in Table 18. A representative chromatogram is shown in Figure 42.

The renal excretion (cumulative, 0-48 h) of the glucuronide and sulfate conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF, expressed as percent of the dose, is shown in Table 19. All subjects showed a decrease in the renal excretion of the 5-hydroxy PF conjugates after phenobarbital treatment. The renal excretion of 5-hydroxy PF conjugates was 13.9% of the dose in the control state and 10.6% of the dose after phenobarbital treatment.

Three subjects (BK, CA and UH) did not show a change while the others showed a minor reduction in the renal excretion of the 5-hydroxy-4-methoxy PF conjugates after phenobarbital treatment. The renal excretion of

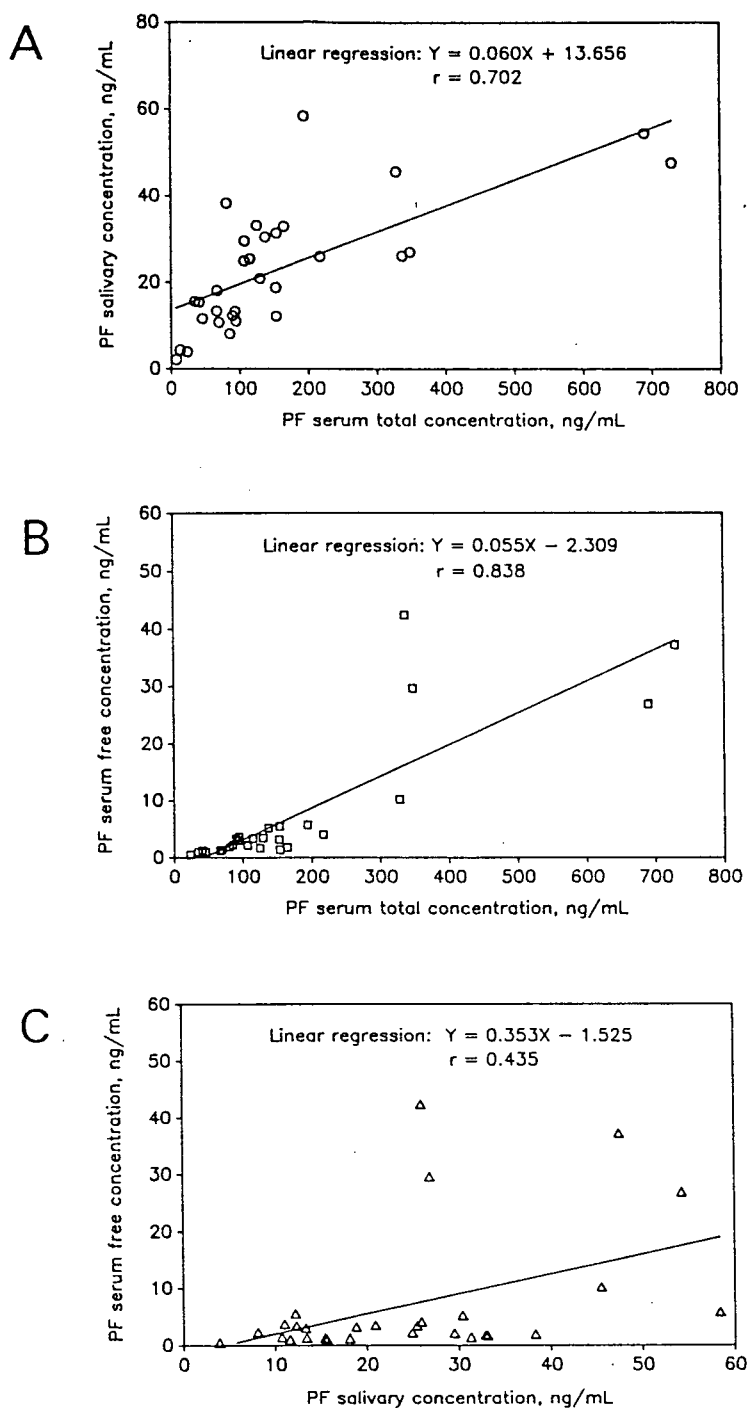


Figure 41. The correlation between (A) PF serum total concentration and salivary concentration (O); (B) PF serum total concentration and serum free concentration (□) and (C) PF salivary concentration and serum free concentration (Δ) in eight healthy non-smokers.

Table 18. Calibration curve data of 5-hydroxy propafenone and 5-hydroxy-4-methoxy propafenone for urine samples.

Number of samples, n = 2 (two injections for each sample)

Concentration of metabolite ($\mu\text{g/mL}$)	Peak area ratio metabolite/I.S.-b (mean \pm s.d.)	Coefficient of variation (%)
5-Hydroxy propafenone		
1.02	0.165 \pm 0.012	7.3
2.03	0.323 \pm 0.009	2.8
4.06	0.625 \pm 0.024	3.8
6.10	0.957 \pm 0.019	2.0
8.13	1.286 \pm 0.025	1.9
10.16	1.627 \pm 0.058	3.6

Statistics: linear regression, $Y = 0.160X - 0.007$; $r = 0.999$

5-Hydroxy-4-methoxy propafenone

1.02	0.129 \pm 0.007	5.4
2.05	0.299 \pm 0.027	9.0
4.09	0.622 \pm 0.033	5.3
6.14	0.959 \pm 0.068	7.1
8.19	1.399 \pm 0.069	4.9
10.24	1.738 \pm 0.110	6.3

Statistics: linear regression, $Y = 0.176X - 0.072$; $r = 0.999$

s.d. standard deviation

C.V. coefficient of variation

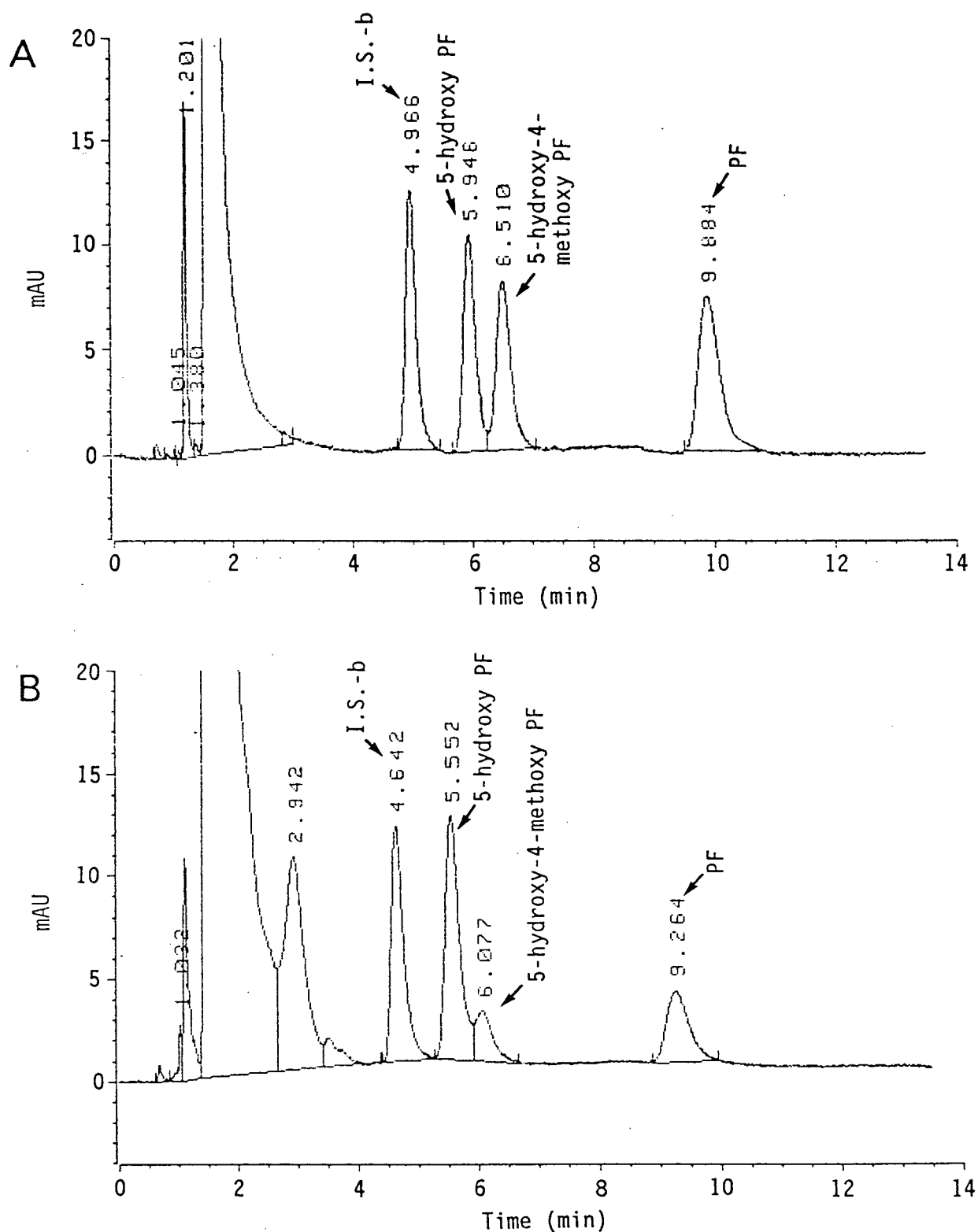


Figure 42. Chromatograms of extract from (A) blank urine spiked with 5-hydroxy propafenone (6 μ g), 5-hydroxy-4-methoxy propafenone (6 μ g), propafenone (6 μ g) and I.S.-b (7 μ g) and (B) a urine sample (spiked with I.S.-b, 7 μ g) from a subject receiving propafenone.

Table 19. Renal excretion of 5-hydroxy propafenone and 5-hydroxy-4-methoxy propafenone conjugates (cumulative) before and after phenobarbital treatment from eight healthy non-smoking subjects.

Subject	Renal excretion, % of dose							
	Control (untreated)				Phenobarbital-treated			
	0-12h	12-24h	24-48h	Total	0-12h	12-24h	24-48h	Total
5-Hydroxy PF conjugates								
BK	11.1	1.8	0.9	13.8	7.4	1.2	1.4	10.0
NP	11.5	0.6	1.2	13.3	10.1	0.4	0.9	11.4
CA	13.5	1.3	0.7	15.5	10.9	1.4	0.8	13.1
DA	11.0	1.0	0.6	12.6	6.0	0.8	0.4	7.2
SG	11.3	0.7	0.4	12.4	10.0	0.6	0.3	10.9
MV	12.9	5.0	2.1	20.0	10.6	2.4	0.3	13.3
GP	9.6	1.0	0.5	11.1	8.0	0.7	0.6	9.3
UH	12.1	0.2	0.2	12.5	9.2	0.1	0.1	9.4
Mean				13.9				10.6
± s.d.				±2.8				±2.0
5-hydroxy-4-methoxy PF conjugates								
BK	2.1	1.0	0.7	3.8	1.7	0.8	1.3	3.9
NP	3.4	0.4	1.0	4.8	2.8	0.4	0.8	4.0
CA	3.5	1.2	0.6	5.3	3.2	1.3	0.8	5.3
DA	3.3	0.9	0.4	4.5	2.2	0.8	0.3	3.3
SG	3.6	0.7	0.3	4.6	2.5	0.4	0.2	3.1
MV	-*	1.2	0.4	1.6	-	1.3	0.2	1.5
GP	2.4	0.9	0.4	3.7	1.8	0.7	0.7	3.2
UH	3.9	0.1	0.1	4.1	3.8	0.2	0.1	4.1
Mean				4.1				3.6
± s.d.				±1.1				±1.1

s.d. standard deviation

* 5-hydroxy-4-methoxy undetected

5-hydroxy-4-methoxy PF conjugates was 4.1% of the dose in the control state and 3.6% of the dose after phenobarbital treatment.

3.7. Phenobarbital Treatment in Healthy Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

3.7.1. Serum Data of Propafenone

The semi-logarithmic plots of serum concentration *versus* time curves of PF from the eight healthy smoking subjects are shown in Appendix 5.

The kinetic data of PF in serum before and after phenobarbital treatment is shown in Table 20. Like the observations noted in the non-smoking subjects, there was a large interindividual variability in all kinetic parameters among smokers. For example, CL_{int} in the control state ranged from 0.4 to 20.7 L/min, with two of the eight smoking subjects (GE and MG) being 'slow' metabolizers ($CL_{int} \leq 0.5$ L/min). Excluding the data of the two 'slow' metabolizers, there was still a large interindividual variation in this parameter, ranging from 8.0 to 20.7 L/min. Besides a smaller CL_{int} , the two 'slow' metabolizers had a longer $t_{1/2\beta}$, a higher C_{max} and a larger AUC when compared to the 'rapid' metabolizers. The mean $t_{1/2\beta}$ of the two 'slow' metabolizers (11.5 h) was 4 times longer than that of the 'rapid' metabolizers (2.7 h). The mean C_{max} of the two 'slow' metabolizers (635 ng/mL) was 6 times higher than that of the 'rapid' metabolizers (107 ng/mL) while the mean AUC of the two 'slow' metabolizers (9975 h.ng/mL) was 25 times larger than that of the 'rapid' metabolizers (405 h.ng/mL). Other kinetic parameters also showed wide intersubject variation, even after the data of the two 'slow' metabolizers were

Table 20. The kinetic data of propafenone in serum before and after phenobarbital treatment from eight healthy smoking subjects.

Subject		$t_{1/2\beta}$ (h)	C_{\max} (ng/mL)	t_{\max} (h)	CL_{int} (L/min)	AUC (h.ng/mL)	% increase in CL_{int}	% decrease in C_{\max}	% decrease in AUC
JL	C	3.2	83	1	13.3	338.6	80	30	44
	P	3.3	58	1.5	23.9	188.8			
MA	C	2.2	222	3	6.4	705.8	23	37	19
	P	2.4	139	1.5	7.9	570.9			
TN	C	1.9	51	4	20.7	218.6	69	20	41
	P	2.3	41	1.5	34.9	129.5			
DW	C	4.5	125	3	8.0	567.3	450	85	82
	P	2.5	19	3	44.0	102.6			
GE	C	11.3	711	3	0.4	11513.1	25	8	22
	P	9.5	651	4	0.5	8932.3			
MG	C	11.6	559	3	0.5	8435.9	100	50	46
	P	12.6	280	4	1.0	4549.3			
SR	C	2.0	64	1	18.2	248.2	142	58	59
	P	1.9	27	1.5	44.0	102.6			
DB	C	2.6	99	2	12.8	353.8	355	80	78
	P	1.8	20	3	58.3	77.5			
Mean \pm s.d.	C	4.9 \pm 4.1	239 \pm 253	2.5 \pm 1.1	10.0 \pm 7.6	2797.7 \pm 4508.2	156 \pm 159	46 \pm 28	49 \pm 23
	P	4.5 \pm 4.1	154 ^S \pm 220	2.5 \pm 1.1	26.8 ^S \pm 21.9	1831.7 ^S \pm 3252.4			
Mean* \pm s.d.	C	2.7 \pm 1.0	107 \pm 62	2.3 \pm 1.2	13.2 \pm 5.6	405.4 \pm 191.4	187 \pm 174	52 \pm 27	54 \pm 24
	P	2.4 \pm 0.5	51 ^S \pm 46	2.0 \pm 0.8	35.5 ^S \pm 17.7	195.3 ^S \pm 187.9			

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

* mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (subject GE and MG)

s.d. standard deviation

excluded. For example, a 4 fold difference in C_{\max} (range 51-222 ng/mL) and a 3 fold difference in AUC (range 218.6-705.8 h.ng/mL) were noted in the control state while a 7 fold difference in C_{\max} (range 19-139 ng/mL) and AUC (range 77.5-570.9 h.ng/mL) were observed after phenobarbital treatment.

There was a significant increase in the apparent CL_{int} of smokers and a significant decrease in C_{\max} and AUC after phenobarbital treatment, including or excluding the data of the two 'slow' metabolizers. The absolute increase in CL_{int} ranged from 23 to 450%, with a mean of 156% (or a mean of 187% excluding the 'slow' metabolizers). The absolute decrease in C_{\max} ranged from 8 to 85%, with a mean of 46% (or a mean of 52% excluding the 'slow' metabolizers) while the decrease in AUC ranged from 19 to 82%, with a mean of 49% (or 54% excluding the 'slow' metabolizers). In addition, the percent decrease in C_{\max} was also not significantly different from the percent decrease in AUC in smokers.

The $t_{1/2\beta}$ of the smokers in the control state ranged from 1.9 to 11.6 h. This large intersubject variation was due to the longer $t_{1/2\beta}$ of the two 'slow' metabolizers (when excluding the 'slow' metabolizers, $t_{1/2\beta}$ ranged from 1.9 to 4.5 h). No significant change in the $t_{1/2\beta}$ was observed after phenobarbital treatment (which ranged from 1.8 to 12.6 h or from 1.8 to 3.3 h excluding the 'slow' metabolizers).

The t_{\max} of PF in the control state ranged from 1 to 4 h. Phenobarbital treatment did not change the t_{\max} (which ranged from 1.5 to 4 h or from 1.5 to 3 h when excluding the 'slow' metabolizers), including or excluding the data of the two 'slow' metabolizers.

Table 21 shows the values of V_{darea}/F and V_{dss}/F of the smokers before and after phenobarbital treatment. In the control state, V_{darea}/F

Table 21. The volume of distribution of propafenone before and after phenobarbital treatment from eight healthy smoking subjects.

Subject		V_{darea}/F (L/kg)*	V_{dss}/F (L/kg)*
JL	C	33.6	40.3
	P	80.9	130.1
MA	C	15.1	20.3
	P	20.8	22.7
TN	C	53.8	88.0
	P	109.4	86.3
DW	C	30.7	34.9
	P	120.7	162.1
GE	C	4.5	4.9
	P	4.9	5.2
MG	C	5.9	6.3
	P	11.7	11.7
SR	C	41.9	44.8
	P	94.8	105.8
DB	C	34.5	41.8
	P	123.5	212.1
Mean \pm s.d.	C	27.5 \pm 17.5	35.2 \pm 26.5
	P	70.8 \pm 50.4 ^S	92.0 \pm 75.4 ^S
Mean \pm s.d.**	C	34.9 \pm 12.8	45.0 \pm 22.8
	P	91.7 \pm 38.3 ^S	119.9 \pm 65.1 ^S

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

* normalized to the individual's body weight

** mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (subjects GE and MG)

s.d. standard deviation

ranged from 4.5 to 53.8 L/kg (or 15.1 to 53.8 L/kg excluding the 'slow' metabolizers) and V_{dss}/F ranged from 4.9 to 88.0 L/kg (or 20.3 to 88.0 L/kg excluding the 'slow' metabolizers). There was a 1 to 5 fold increase in both V_{darea}/F and V_{dss}/F after phenobarbital treatment, the difference being statistically significant. The values of V_{dss}/F were not significantly different from the values of V_{darea}/F in smokers.

Although the interindividual variations in C_{max} , CL_{int} and AUC were reduced substantially when the data from the two 'slow' metabolizers were excluded, there was still a wide range in the percent increase in CL_{int} and the percent decrease in C_{max} and AUC.

3.7.2. Salivary Data of Propafenone

The salivary concentration *versus* time curves of PF obtained before and after phenobarbital treatment from the eight smoking subjects are shown in Appendix 6. Like the non-smokers, the smokers also showed fluctuations in salivary PF concentrations, yet the PF salivary concentrations were reduced after phenobarbital treatment.

The kinetic data of PF in saliva before and after phenobarbital treatment is shown in Table 22. The saliva/serum PF concentration ratios were 0.36 ± 0.20 (or 0.33 ± 0.22 excluding the two 'slow' metabolizers) in the control state and 0.40 ± 0.15 (or 0.40 ± 0.18 excluding the two 'slow' metabolizers) after phenobarbital treatment, with large inter- and intraindividual variation. In two subjects, the saliva/serum PF concentration ratio ranged from 0.04 to 0.18 (subject MA, control) and 0.06 to 1.06 (subject DB, phenobarbital-treated). The saliva/serum PF concentration ratios were inversely correlated with the pH of saliva in both the control state ($r = -0.541$, $n = 80$, $p < 0.05$) and after phenobarbital

Table 22. The kinetic data of propafenone in saliva before and after phenobarbital treatment from eight healthy smoking subjects.

Subject		saliva/serum PF concentration ratio			AUC ₀ ^t (h.ng/mL)	% decrease in AUC ₀ ^t
JL	C	0.08	±	0.09	16.9	-
	P	0.21	±	0.25	24.8	
TN	C	0.37	±	0.09	72.4	30
	P	0.58	±	0.36	50.9	
MA	C	0.10	±	0.05	64.5	-
	P	0.26	±	0.13	156.7	
DW	C	0.66	±	0.33	353.0	81
	P	0.63	±	0.31	67.7	
GE	C	0.53	±	0.20	6588.2	34
	P	0.43	±	0.19	4378.1	
MG	C	0.36	±	0.14	3199.3	35
	P	0.40	±	0.25	2093.3	
SR	C	0.35	±	0.16	82.5	69
	P	0.25	±	0.15	25.5	
DB	C	0.44	±	0.22	118.3	77
	P	0.44	±	0.38	27.2	
Mean ± s.d.	C	0.36			1311.9	54
		±0.20			±2391.3	±24
	P	0.40			853.0	
		±0.15			±1592.9	
Mean ^{**} ± s.d.	C	0.33			117.9	64
		±0.22			±119.7	±23
	P	0.40			58.8	
		±0.18			±51.0	

C control (untreated)

P phenobarbital-treated

* subjects (JL and MA) who did not show a decrease in salivary AUC after phenobarbital treatment, their data excluding from the calculation of the mean % decrease in AUC

** mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (subjects GE and MG)

s.d. standard deviation

treatment ($r = -0.387$, $n = 72$, $p < 0.05$). The best fit through the data points are $Y = 7.000 - 0.630X$ (control) and $Y = 6.868 - 0.464X$ (phenobarbital-treated). The two 'slow' metabolizers also had a higher salivary AUC_0^t compared to the 'rapid' metabolizers. The mean AUC_0^t of the two 'slow' metabolizers (4894 h.ng/mL) was 41 times higher than that of the 'rapid' metabolizers (118 h.ng/mL). Subjects JL and MA showed an increase in salivary AUC_0^t after phenobarbital treatment. Although the change in AUC_0^t was not statistically significant, the absolute decrease in AUC_0^t (excluding subjects JL and MA) ranged from 30 to 81%, with a mean of 54% (or 64% excluding the two 'slow' metabolizers).

3.7.3. Serum Data of 5-Hydroxy Propafenone

The serum concentration *versus* time curves of 5-hydroxy PF from the eight smoking subjects are shown in Appendix 14-15. The serum concentrations of 5-hydroxy PF were also lower than the serum concentrations of PF in smokers.

The kinetic data of 5-hydroxy PF in serum is shown in Table 23. The metabolite, 5-hydroxy PF, was quantitated in 6 smokers. Subject GE and MG had undetectable levels of 5-hydroxy PF in their serum, which is a unique characteristic of 'slow' metabolizers. Even after phenobarbital treatment, these two subjects still had undetectable quantities of serum 5-hydroxy PF. There was also a large intersubject variation in the kinetic parameters of 5-hydroxy PF. For example, a two fold difference in both C_{max} (range 77-165 ng/mL, mean 122 ng/mL) and AUC (range 310.5-673.3 h.ng/mL, mean 460.3 h.ng/mL) was noted among the 'rapid' metabolizers in the control state. There were significant decreases in both C_{max} and AUC of 5-hydroxy PF after phenobarbital treatment. The absolute decrease in C_{max} ranged

Table 23. The kinetic data of 5-hydroxy propafenone in serum before and after phenobarbital treatment from six healthy smoking subjects.

Subject		$t_{1/2\beta}$ (h)	C_{\max} (ng/mL)	t_{\max} (h)	AUC (h.ng/mL)	% decrease in C_{\max}	% decrease in AUC
JL	C	5.1	89	1	321.1	37	48
	P	5.3	56	1.5	168.5		
MA	C	5.0	165	3	673.3	61	56
	P	6.9	65	1.5	295.2		
TN	C	2.9	77	3	364.3	19	67
	P	1.2	62	1.5	118.4		
DW	C	2.7	153	3	596.3	71	72
	P	2.2	44	3	167.0		
SR	C	4.0	108	1	310.5	54	49
	P	2.9	50	1.5	159.2		
DB	C	3.6	137	1.5	496.4	68	66
	P	2.0	44	3	169.8		
Mean*		3.9	122	2.1	460.3	52	60
\pm s.d.		± 1.0	± 36	± 1.0	± 152.4	± 20	± 10
P		3.4	54 ^S	2.0	179.7 ^S		
		± 2.2	± 9	± 0.8	± 59.8		

C control (untreated)

P phenobarbital-treated

S Wilcoxon paired-sample test, compared to the control, significant, $p < 0.05$

s.d. standard deviation

* mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (subjects GE and MG). Their serum 5-hydroxy PF concentrations were lower than the detection limit.

from 19 to 71%, with a mean of 52% while the decrease in AUC ranged from 48 to 72%, with a mean of 60%. The percent decrease in C_{\max} was not significantly different from the percent decrease in AUC.

The $t_{1/2\beta}$ ranged from 2.7 to 5.1 h (mean 3.9 h) in the control state. No significant change was observed after phenobarbital treatment (range 1.2-6.9 h, mean 2.1 h).

The t_{\max} of 5-hydroxy PF ranged from 1 to 3 h (mean 2.1 h) in the control state. Phenobarbital treatment did not change the t_{\max} (range 1.5-3 h, mean 2.0 h).

3.7.4. Protein Binding and Serum α_1 -Acid Glycoprotein Concentration

The free fraction of PF (2 and 4 h samples) was 0.027 ± 0.008 in the control state and 0.030 ± 0.004 after phenobarbital treatment. Serum AAG concentrations in the smokers ranged from 57.7 to 113.0 mg/dL (mean 79.1 mg/dL) in the control state and 61.5 to 122.0 mg/dL (mean 80.1 mg/dL) after phenobarbital treatment. Twenty-three days of phenobarbital administration did not cause any significant change in either PF free fraction (Figure 43) or serum AAG concentration (Figure 44).

Table 24 shows the data of the saliva/serum PF concentration ratios and salivary pH of the 2 and 4 h samples and allows comparison of the values of percent of free PF in these samples obtained by two different methods. The percent of free PF in serum, calculated from the data of PF serum/saliva concentration ratio, pH of saliva and pK_a of PF using Equation 7, was significantly higher than the values obtained from equilibrium dialysis.

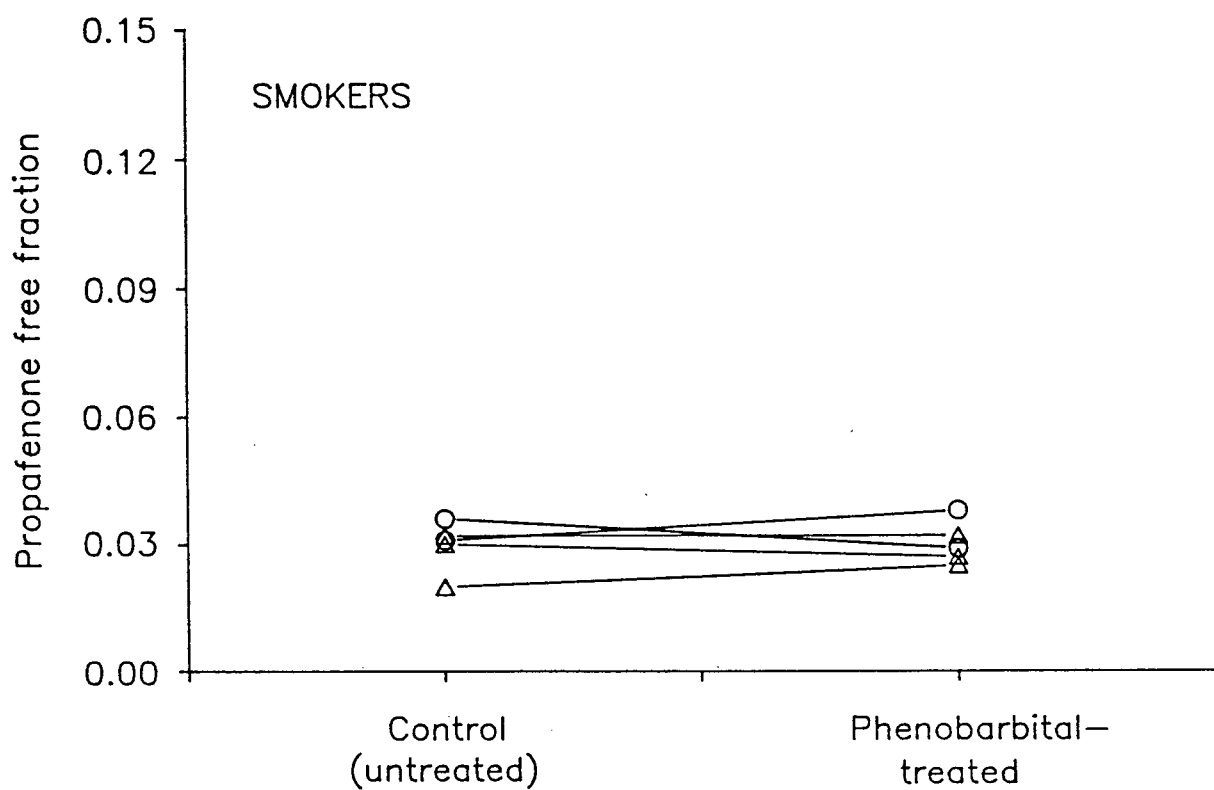


Figure 43. The free fraction of propafenone [2 (○) and 4 (Δ) h samples] before and after 23 days of phenobarbital treatment in eight healthy smokers.

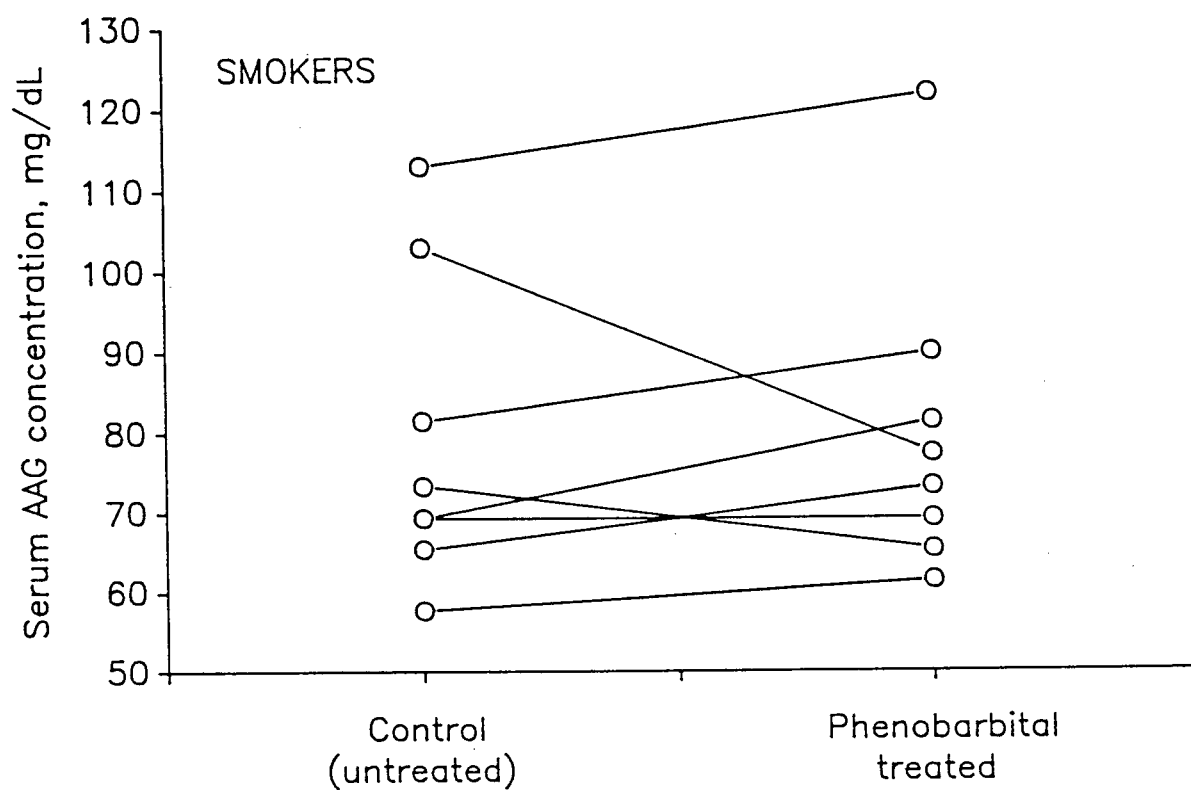


Figure 44. The serum AAG concentration (0 h sample) before and after 23 days of phenobarbital treatment in eight healthy smokers.

Table 24. The saliva/serum concentration ratio of propafenone, pH of saliva and percent of free PF in serum from eight healthy smoking subjects.

Subject		Sampling time (h)	Propafenone concentration ratio (saliva/serum)	pH of saliva	Serum protein binding (% free)	
					observed ^a	calculated ^b
JL	C	2	0.04	7.39	3.1	3.9
		4	0.03	7.71	4.1	6.0
	P	2	0.09	7.40	3.8	9.0
		4	0.09	7.45	-*	10.1
MA	C	2	0.07	6.90	1.9	2.3
		4	0.10	6.96	2.0	3.7
	P	2	0.38	6.46	-	4.5
		4	0.36	6.65	2.5	6.5
TN	C	2	0.21	6.14	-	1.2
		4	0.42	6.60	2.4	6.8
	P	2	0.39	6.57	2.7	5.9
		4	0.48	6.40	-	4.9
DW	C	2	0.29	6.47	3.1	3.5
		4	0.75	6.46	3.0	8.8
	P	2	0.60	6.46	-	7.0
		4	0.83	6.75	-	18.9
GE	C	2	0.44	6.57	3.6	6.6
		4	0.53	6.65	3.2	9.6
	P	2	0.22	6.83	2.9	6.0
		4	0.54	6.84	3.2	15.1
MG	C	2	0.29	6.68	3.6	5.6
		4	0.65	6.57	3.0	9.8
	P	2	0.44	6.70	2.9	9.0
		4	0.58	6.68	2.7	11.3
SR	C	2	0.33	7.00	2.4	13.3
		4	0.39	7.12	1.3	20.7
	P	2	0.49	6.56	-	-
		4	0.11	6.70	-	-
DB	C	2	0.16	6.72	2.6	3.4
		4	0.70	6.51	1.4	9.2
	P	2	0.12	6.46	-	-
		4	0.37	6.46	-	-

C control (untreated)

P phenobarbital-treated

a obtained from equilibrium dialysis, n=3

b calculated using equation 7

Wilcoxon paired-sample test, compared to the observed values, significant, p<0.05

* values unavailable

3.7.5. Relationship Between Serum Total, Serum Free and Salivary Propafenone Concentrations

Figure 45 shows the relationship between PF serum total concentration, serum free concentration and salivary concentration of the 2 and 4 h samples (control and phenobarbital) in the smoking subjects. The linear regression and correlations are described as follows: PF serum total concentration and PF salivary concentration, $Y = 0.495X - 9.907$, $r = 0.944$ ($n = 32$, $p < 0.05$); PF serum total concentration and PF serum free concentration, $Y = 0.033X - 0.453$, $r = 0.994$ ($n = 22$, $p < 0.05$); PF salivary concentration and PF serum free concentration, $Y = 0.056X + 1.317$, $r = 0.924$ ($n = 22$, $p < 0.05$).

3.7.6. Urinary Data

The renal excretion (cumulative, 0-48 h) of the glucuronide and sulfate conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF, expressed as percent of the dose, is shown in Table 25. Subject TN missed some urine samples and his urinary data were excluded. Subject GE did not show any change in the renal excretion of the 5-hydroxy PF conjugates while the remaining subjects showed a reduction after phenobarbital treatment. The renal excretion of 5-hydroxy PF conjugates was 10.7% of dose in the control state and 8.0% after phenobarbital treatment.

Subjects GE and MG had undetectable levels of 5-hydroxy-4-methoxy PF conjugates in their urine. Subject SR did not show a change while the remaining subjects showed a reduction in the renal excretion of the 5-hydroxy-4-methoxy PF conjugates. The renal excretion of 5-hydroxy-4-methoxy PF conjugates was 5.4% of dose in the control state and 4.6% of dose after phenobarbital treatment.

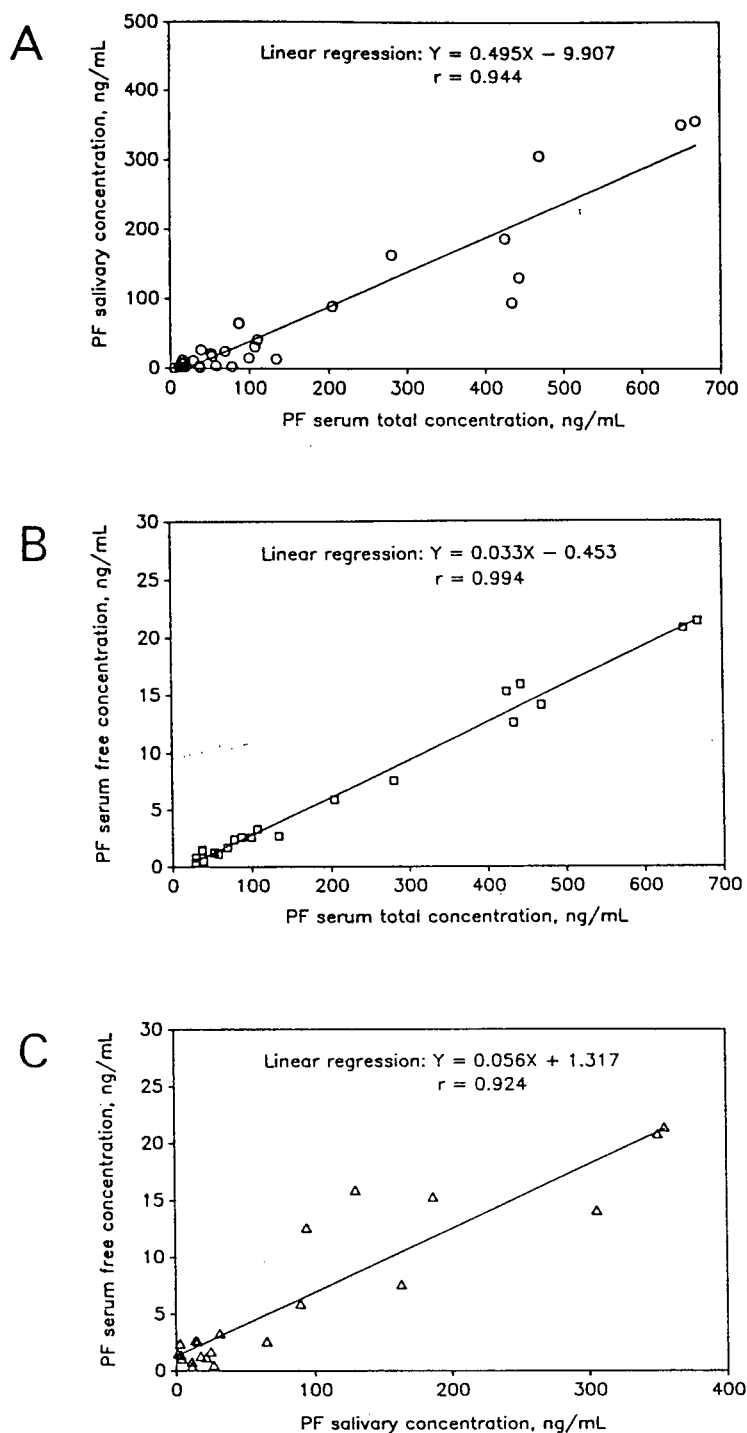


Figure 45. The correlation between (A) PF serum total concentration and salivary concentration (○); (B) PF serum total concentration and serum free concentration (□) and (C) PF salivary concentration and serum free concentration (Δ) in eight healthy smokers.

Table 25. Renal excretion of 5-hydroxy propafenone and 5-hydroxy-4-methoxy propafenone conjugates (cumulative) before and after phenobarbital treatment from eight healthy smoking subjects.

Subject	Renal excretion, % of dose							
	Control (untreated)				Phenobarbital-treated			
	0-12h	12-24h	24-48h	Total	0-12h	12-24h	24-48h	Total
5-Hydroxy PF conjugates								
JL	15.8	1.0	1.3	18.1	10.7	0.6	0.7	12.0
MA	16.3	0.6	0.3	17.2	13.8	0.6	0.3	14.7
TN ^a	-*	-	-	-	-	-	-	-
DW	10.0	0.2	0.4	10.6	5.7	0.4	0.2	6.3
GE ^b	2.2	0.4	1.1	3.7	2.5	0.5	0.7	3.7
MG ^b	3.3	0.8	0.8	4.9	3.1	0.5	1.0	4.6
SR	6.1	0.1	-	6.2	4.3	0.04	-	4.3
DB	13.8	0.4	-	14.2	10.4	0.3	-	10.7
Mean				10.7				8.0
± s.d.				±5.9				±4.4
5-hydroxy-4-methoxy PF conjugates								
JL	4.1	0.8	0.7	5.6	3.5	0.6	0.5	4.6
MA	4.8	0.6	0.4	5.8	3.3	0.6	0.2	4.1
TN ^a	-	-	-	-	-	-	-	-
DW	3.1	0.1	0.4	3.6	2.1	0.8	0.3	3.2
GE ^b	-	-	-	-	-	-	-	-
MG ^b	-	-	-	-	-	-	-	-
SR	3.4	0.2	-	3.6	3.1	0.4	-	3.5
DB	7.3	1.0	-	8.3	6.0	1.3	0.2	7.5
Mean				5.4				4.6
± s.d.				±1.9				±1.7

a subject TN missed a urine sample and subjects GE and MG had
b undetectable concentrations of 5-hydroxy-4-methoxy PF conjugates in
their urine
* values unavailable
s.d. standard deviation

3.8. Phenobarbital Treatment: Comparison of Effect on Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone Between Healthy Non-smokers and Smokers

Table 26 allows comparison of the pharmacokinetic parameters of PF in serum before and after phenobarbital treatment between non-smoking and smoking subjects. Compared to the non-smokers, the smokers (with the exclusion of the data of the two 'slow' metabolizers) had a significantly larger CL_{int} , a smaller C_{max} and AUC and a larger V_{darea}/F and V_{dss}/F in the control state. When the values of $t_{1/2\beta}$ and t_{max} were compared, there was no significant difference between these two groups, including or excluding the data of the two 'slow' metabolizers from the smoking group. No significant difference was observed when the kinetic parameters were compared across groups after the phenobarbital test. However, when the control values for AUC and C_{max} were compared in the non-smoking and smoking groups, phenobarbital was found to cause a significant increase in CL_{int} ($194 \pm 266\%$ in the non-smokers and $156 \pm 159\%$ in the smokers) and a significant reduction in both AUC ($50 \pm 26\%$ in the non-smokers and $49 \pm 23\%$ in the smokers) and C_{max} ($45 \pm 27\%$ in the non-smokers and $46 \pm 28\%$ in the smokers) of PF. Serum AAG concentrations in both non-smoking and smoking subjects were within the normal range and there was no significant difference in this parameter between the two groups. There was also no significant difference in the free fraction of PF between the non-smokers and the smokers.

Table 27 shows comparison of the pharmacokinetic parameters of 5-hydroxy PF in serum before and after phenobarbital treatment between eight non-smoking and six smoking subjects. When the values of the kinetic

Table 26. Comparison of pharmacokinetic parameters of propafenone in serum between eight non-smokers and eight smokers before and after phenobarbital treatment (all data presented as mean + s.d.).

Kinetic parameter	Non-smokers (n=8)		Smokers (n=8)	
	Control	Phenobarbital-treated	Control	Phenobarbital-treated
$t_{1/2\beta}$ (h)	2.8 ± 1.2	2.5 ± 1.2	4.9 ± 4.1 (2.7 ± 1.0)*	4.5 ± 4.1 (2.4 ± 0.5)
C_{max} (ng/mL)	253 ± 221	137 ± 127	239 ± 253 (107 ± 62) ^S	154 ± 220 (51 ± 46)
t_{max} (h)	2.5 ± 0.9	2.9 ± 1.2	2.5 ± 1.1 (2.3 ± 1.2)	2.5 ± 1.1 (2.0 ± 0.8)
CL_{int} (L/min)	6.1 ± 3.7	18.1 ± 21.5	10.0 ± 7.6 (13.2 ± 5.6) ^S	26.8 ± 21.9 (35.5 ± 17.7)
AUC (h.ng/mL)	1360 ± 1684	638 ± 778	2798 ± 4508 (405 ± 191) ^S	1832 ± 3252 (195 ± 188)
V_{darea}/F (L/kg)**	16.2 ± 12.4	42.3 ± 60.2	27.5 ± 17.5 (34.9 ± 12.8) ^S	70.8 ± 50.4 (91.7 ± 38.3)
V_{dss}/F (L/kg)**	20.0 ± 13.6	60.7 ± 80.7	35.2 ± 26.5 (45.0 ± 22.8) ^S	92.0 ± 75.4 (119.9 ± 65.1)
Serum AAG concentration (mg/dL) ^a	81.1 ± 19.1	77.7 ± 14.2	79.1 ± 19.3	80.1 ± 19.2
PF free fraction ^b	0.027 ± 0.011	0.038 ± 0.032	0.027 ± 0.008	0.030 ± 0.004
% increase in CL_{int}	194 ± 266		156 ± 159 (187 ± 174)	
% decrease in C_{max}	45 ± 27		46 ± 28 (52 ± 27)	
% decrease in AUC	50 ± 26		49 ± 23 (54 ± 24)	

s.d. standard deviation

a AAG concentration of 0 h (blank) serum sample

b the free fraction of PF was determined in the 2 and 4 h serum samples from each subject by equilibrium dialysis

* numbers in brackets are mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers.

** normalized to the individual's body weight

S Mann-Whitney test, compared to the control values of the non-smokers, significant, $p < 0.05$

Table 27. Comparison of pharmacokinetic parameters of 5-hydroxy propafenone in serum between eight non-smokers and six smokers before and after phenobarbital treatment (all data presented as mean \pm s.d.).

Kinetic parameter	Non-smokers (n=8)		Smokers (n=6)*	
	Control	Phenobarbital-treated	Control	Phenobarbital-treated
$t_{1/2\beta}$ (h)	3.1 \pm 1.1	2.9 \pm 1.1	3.9 \pm 1.0	3.4 \pm 2.2
C_{\max} (ng/mL)	141 \pm 62	86 \pm 47	122 \pm 36	54 \pm 9
t_{\max} (h)	2.8 \pm 1.5	2.8 \pm 1.1	2.1 \pm 1.0	2.0 \pm 0.8
AUC (h.ng/mL)	685 \pm 324	387 \pm 234	460 \pm 152	180 \pm 60
% decrease in C_{\max}	40 \pm 22		52 \pm 20	
% decrease in AUC	45 \pm 18		60 \pm 10	

s.d. standard deviation

* mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (subjects GE and MG). Their serum 5-hydroxy PF concentrations were lower than the detection limit.

parameters AUC, C_{\max} , $t_{1/2\beta}$ and t_{\max} measured in the control state were compared between the non-smoking and smoking subjects, there was no significant difference between these two groups. Similar results were observed when these parameters were compared across groups after phenobarbital administration. However, when the control values for AUC and C_{\max} were compared to the treated values in the non-smoking and smoking subjects, phenobarbital caused a significant reduction in both AUC ($45 \pm 18\%$ in the non-smokers and $60 \pm 10\%$ in the smokers) and C_{\max} ($40 \pm 22\%$ in the non-smokers and $52 \pm 20\%$ in the smokers).

The correlation between phenobarbital serum concentration and the extent of metabolic induction of PF by phenobarbital, as expressed by $[(AUC_P - AUC_C)/AUC_C] \times 100\%$, is shown in Figure 46. There was no correlation between these two parameters ($r = -0.323$, $n = 16$, $p > 0.05$). The best fit through the data points was described by $Y = 98.981 - 3.007X$. Figure 47 illustrates the correlation between $CL_{\text{int control}}$ and $[(AUC_P - AUC_C)/AUC_C] \times 100\%$. No significant correlation was observed between these two parameters ($r = 0.048$, $n = 16$, $p > 0.05$). The best fit through the data points was described by $Y = 0.187X + 47.929$.

3.9. Evaluation of Propafenone Serum Concentration-Response Relationship

The semi-logarithmic plots of the serum concentration *versus* time curves of PF and 5-hydroxy PF from the ten patients are shown in Appendix 8.

The kinetic data of PF from the ten patients is shown in Table 28. Two of the ten patients (GB and BM) were 'slow' metabolizers

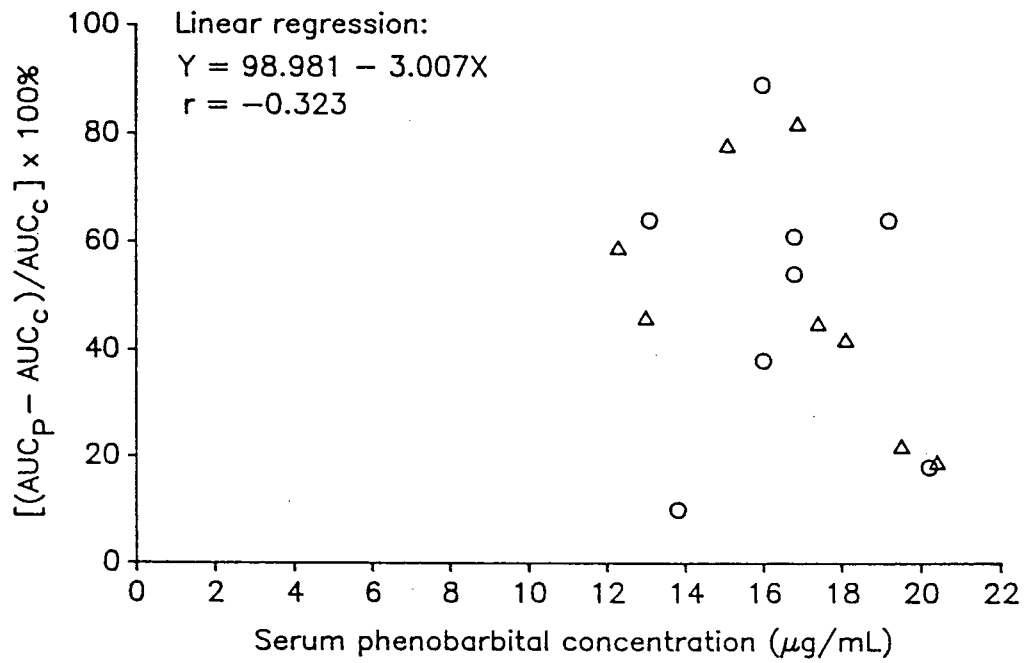


Figure 46. The correlation between phenobarbital serum concentration (day 29) and $[(AUC_P - AUC_C)/AUC_C] \times 100\%$ in eight healthy non-smokers (\circ) and eight healthy smokers (Δ).

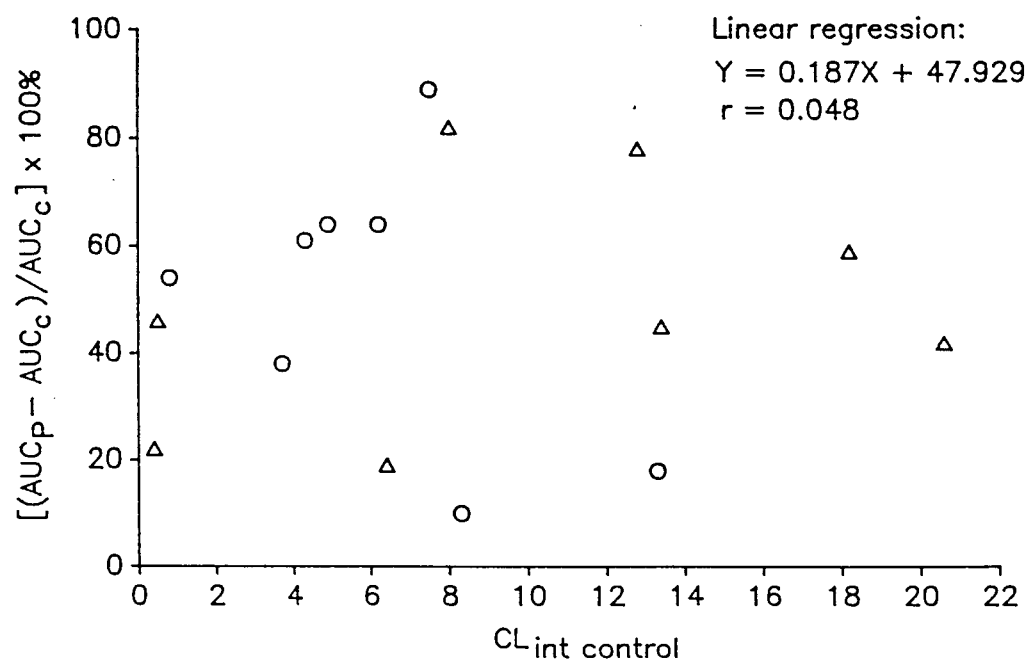


Figure 47. The correlation between values of CL_{int control} and [(AUC_P - AUC_C) / AUC_C] × 100% in eight healthy non-smokers (○) and eight healthy smokers (△).

Table 28. The kinetic data of propafenone and 5-hydroxy propafenone in serum from ten patients.

Patient	PF			5-hydroxy PF
	AUC_0^T (h.ng/mL)	CL_{int} (L/min)	C_{pss} (ng/mL)	AUC_0^T (h.ng/mL)
BN	7329.6	0.62	916	1161.7
C	3916.6	1.15	490	1142.0
JC*	5185.1	0.87	432	1482.7
JL*	2416.7	0.93	403	1575.0
DT*	4441.2	1.02	555	836.9
LS*	2258.9	1.00	282	1162.4
GB	14445.5	0.31	1806	-**
RM	5157.4	0.88	645	795.0
AP	1374.0	3.29	172	510.4
BM	14617.9	0.31	1827	-
Mean ± s.d.	6114.3 ±4756.4	1.04 ±0.84	753 ±596	
Mean [#] ± s.d.	4009.9 ±1944.4	1.22 ±0.85	487 ±228	1083.3 ±356.4

* the predose serum PF concentrations of these patients were used as the serum PF concentrations at the dosing interval in the estimation of AUC_0^T and CL_{int}

** subjects GB and BM had undetectable serum 5-hydroxy propafenone

mean and s.d. of the kinetic parameters excluding the data of the two 'slow' metabolizers (patients GB and BM)

($CL_{int} \leq 0.5$ L/min). In addition to low CL_{int} values, the two 'slow' metabolizers exhibited high Cp_{ss} and large AUC_0^T values. The mean Cp_{ss} (1816.7 ng/mL) and AUC_0^T (14531.7 h.ng/mL) values for the two 'slow' metabolizers were about four times the mean Cp_{ss} (487 ng/mL) and AUC_0^T (4009.9 h.ng/mL) values of the 'rapid' metabolizers. The 'slow' metabolizers account for some of the considerable interindividual variation seen in PF kinetic parameters among patients receiving the drug. However, a large interindividual difference in the kinetic parameters still exists among the 'rapid' metabolizers. There was a 5 fold difference in AUC_0^T (range 1374.0-7329.6 h.ng/mL), CL_{int} (range 0.62-3.29 L/min) and Cp_{ss} (range 172-916 ng/mL) among these patients.

The kinetic data of 5-hydroxy PF from the ten patients is also shown in Table 28. The metabolite, 5-hydroxy PF, was measurable in serum of only eight of the ten patients receiving the drug during treatment for arrhythmias. Serum 5-hydroxy PF concentrations were lower than serum PF concentrations. There was a 3 fold difference in the AUC_0^T (range 510-1575 h.ng/mL) among the patients. Subjects GB and BM had undetectable quantities of 5-hydroxy PF, a characteristic of 'slow' metabolizers.

Figure 48 shows log PF serum concentration *versus* QRS width. The best fit through the data points by linear regression is $Y = 5.094X + 94.249$ ($r = 0.461$, $n = 56$). Figure 49 shows log 5-hydroxy PF serum concentration *versus* QRS width. The best fit by linear regression is $Y = 10.534X + 85.718$ ($r = 0.527$, $n = 45$). QRS width is significantly correlated to either PF serum concentration or 5-hydroxy PF serum concentration.

Figure 50A and B allow comparison of the correlation between log PF serum total concentration and QRS width ($Y = 4.367X + 95.929$, $r = 0.288$,

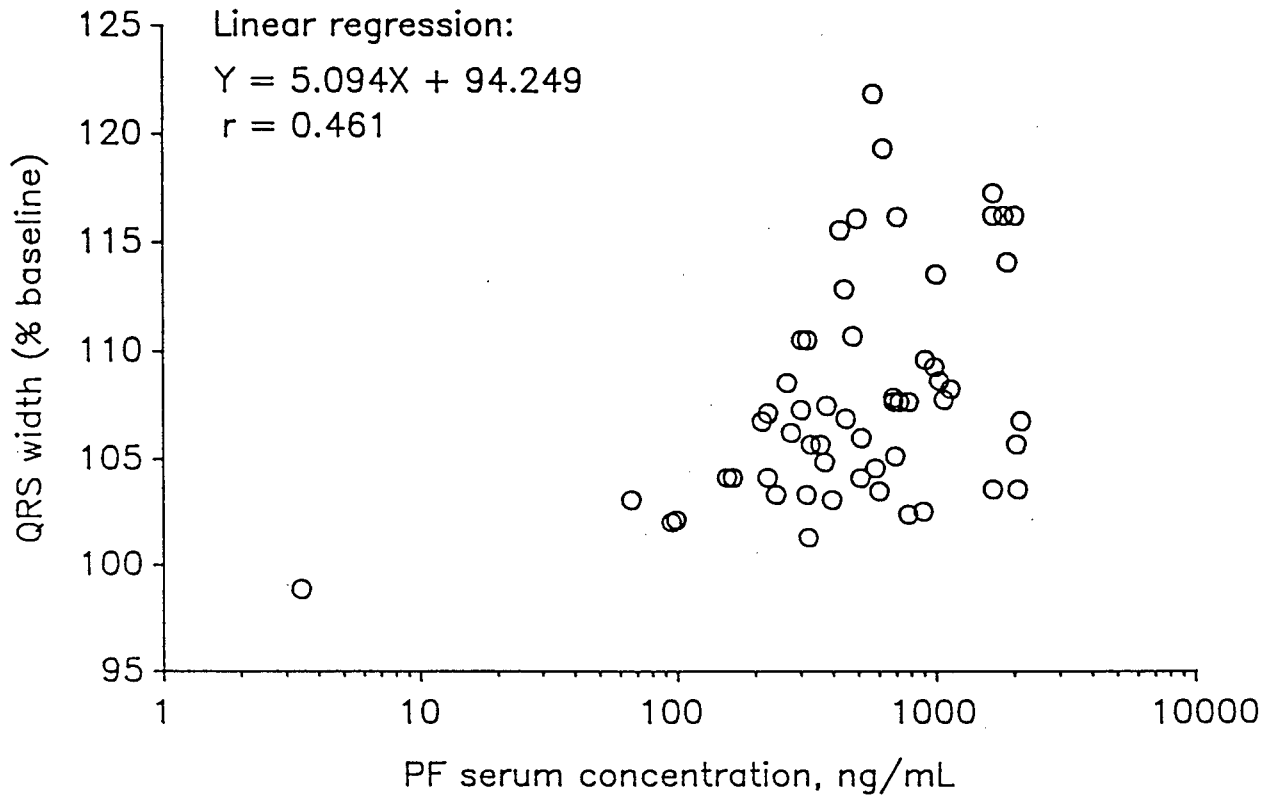


Figure 48. The correlation between QRS width and log PF serum concentration of ten patients.

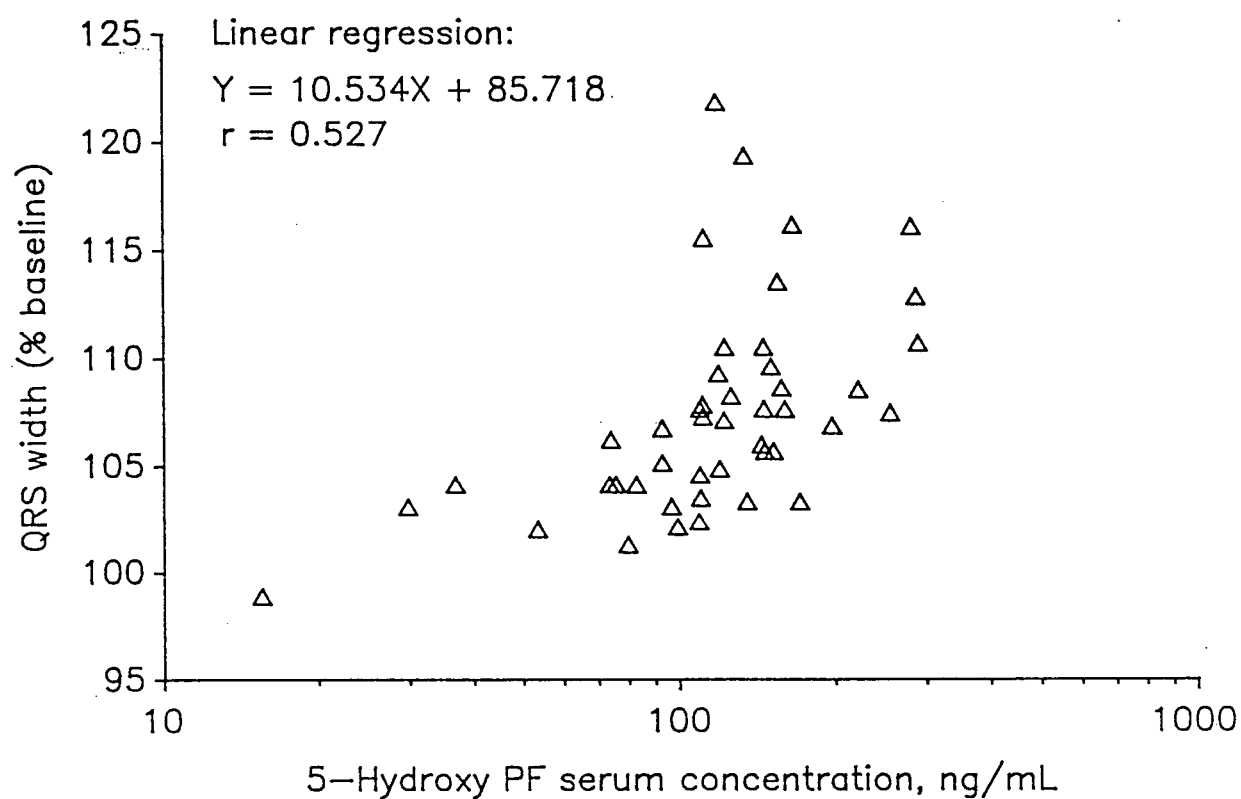


Figure 49. The correlation between QRS width and log 5-hydroxy PF serum concentration of ten patients.

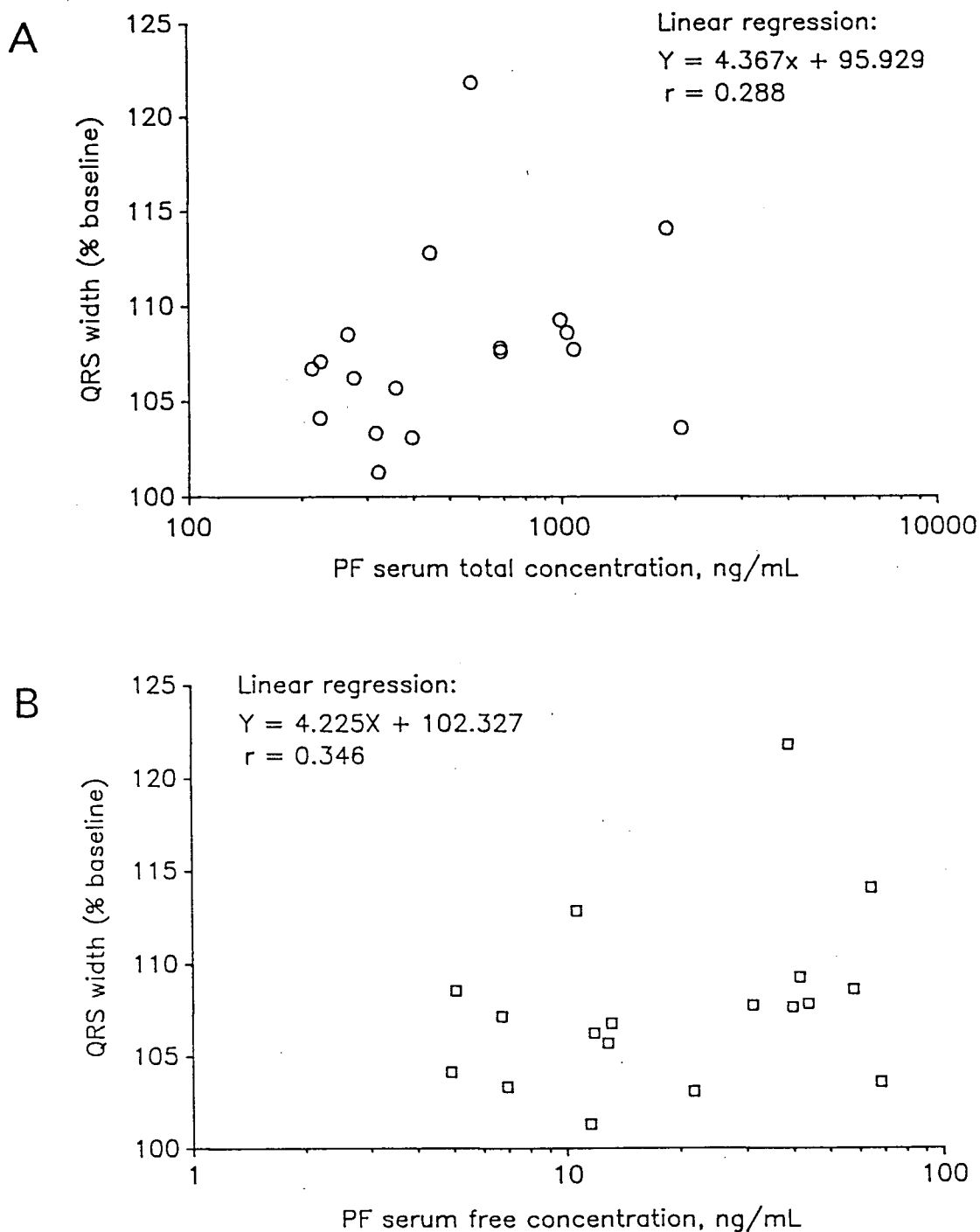


Figure 50. The correlation between (A) QRS width and log PF serum total concentration and (B) QRS width and log PF serum free concentration of ten patients (two data points from each patient).

$n = 18$) and log PF serum free concentration and QRS width ($Y = 4.225X + 102.327$, $r = 0.346$, $n = 18$) (two data points from each patient). QRS width is not significantly ($p > 0.05$) correlated to either PF serum total concentration or PF serum free concentration.

The serum AAG concentrations of the patients ranged from 43 to 115 mg/dL, which is within the normal range of serum AAG concentration. Figure 51 shows the relationship between serum AAG concentration and PF free fraction. The best fit line by linear regression is $Y = -0.0004X + 0.070$. The correlation between serum AAG concentration and PF free fraction is negative and significant ($r = -0.504$, $n = 20$, $p < 0.05$). The non-linear least squares method gives a better fit as $Y = 1/(1 + 0.357X)$.

Using multiple stepwise regression, Equations 8 and 9 are obtained which modelled QRS width as a function of PF serum concentration, 5-hydroxy PF serum concentration and serum AAG concentration:

$$Y = 0.5X_1 + 4.5X_2 + 347X_3 + 79 \quad (8)$$

$$Y = 0.004X_1' + 0.04X_2' + 379X_3 + 95 \quad (9)$$

where Y is QRS width, X_1 is log PF serum concentration, X_2 is log 5-hydroxy PF serum concentration, X_3 is the reciprocal of serum AAG concentration ($1/\text{AAG}$), X_1' is PF serum concentration and X_2' is 5-hydroxy PF serum concentration.

Figure 52 shows the relationship between measured QRS width and predicted QRS width using Equation 8. The correlation is significant ($r = 0.536$, $n = 45$, $p < 0.05$). The best fit line by linear regression is $Y = 0.177X + 74.881$. Figure 53 shows the relationship between measured QRS width and predicted QRS width using Equation 9. The correlation is significant ($r = 0.630$, $n = 45$, $p < 0.05$). The best fit line by linear regression is $Y = 0.387X + 65.902$.

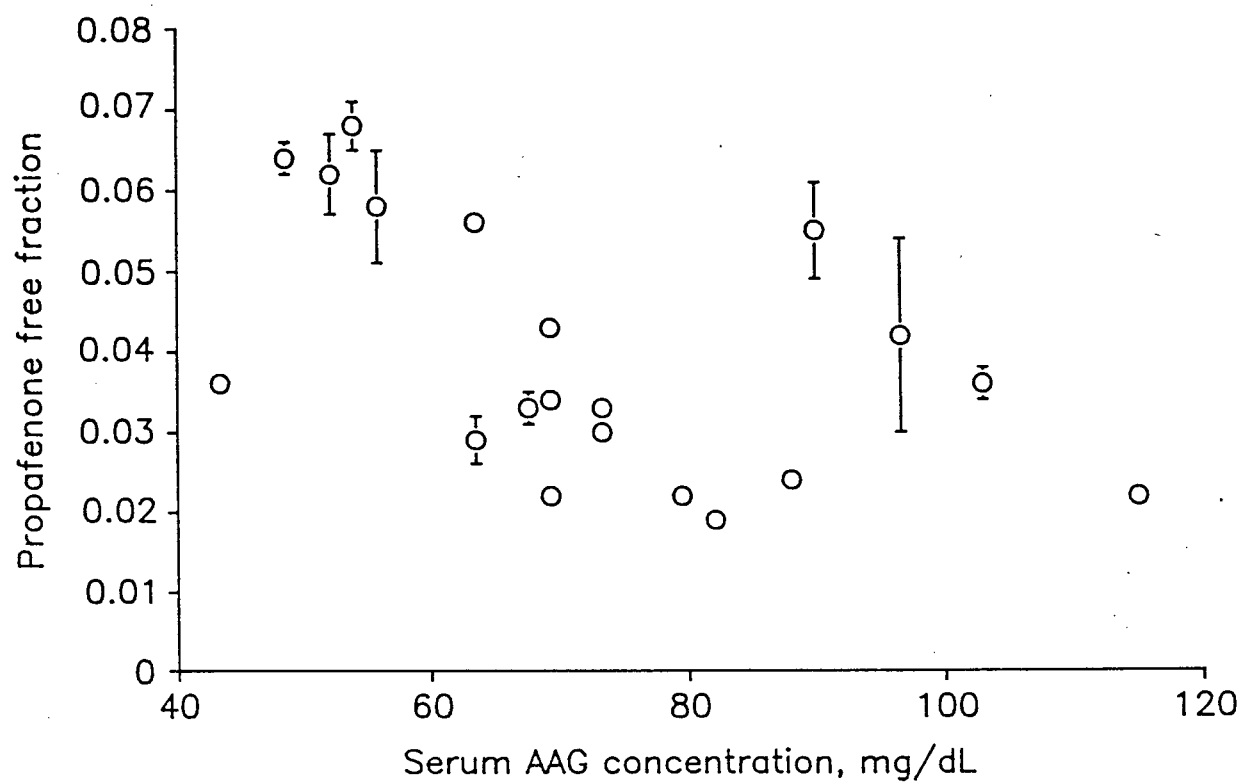


Figure 51. The correlation between serum propafenone free fraction and serum AAG concentration of ten patients. The data are presented as mean \pm 1s.d.

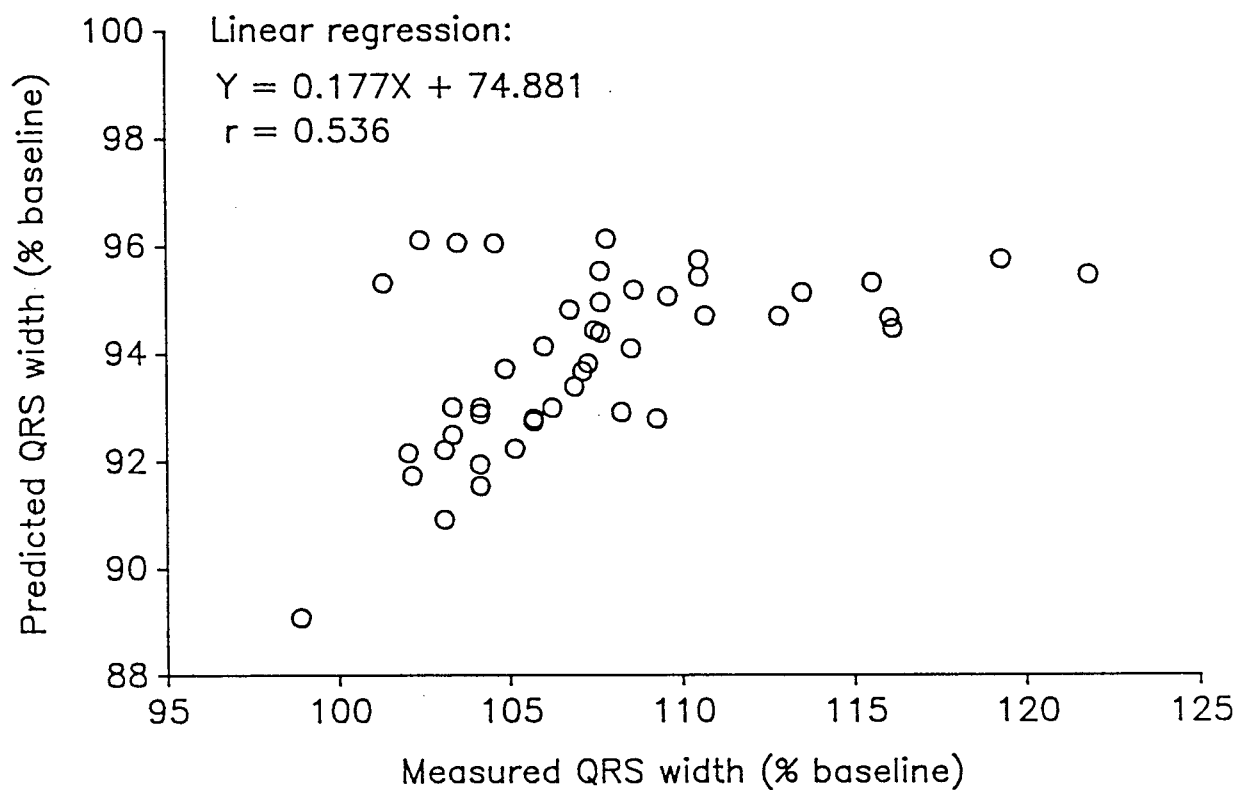


Figure 52. The correlation between measured QRS width and predicted QRS width. QRS width is predicted from the following equation (obtained by multiple stepwise regression): $Y = 0.5X_1 + 4.5X_2 + 347X_3 + 79$ where Y is QRS width, X_1 is log PF serum concentration, X_2 is log 5-hydroxy PF serum concentration and X_3 is $1/AAG$.

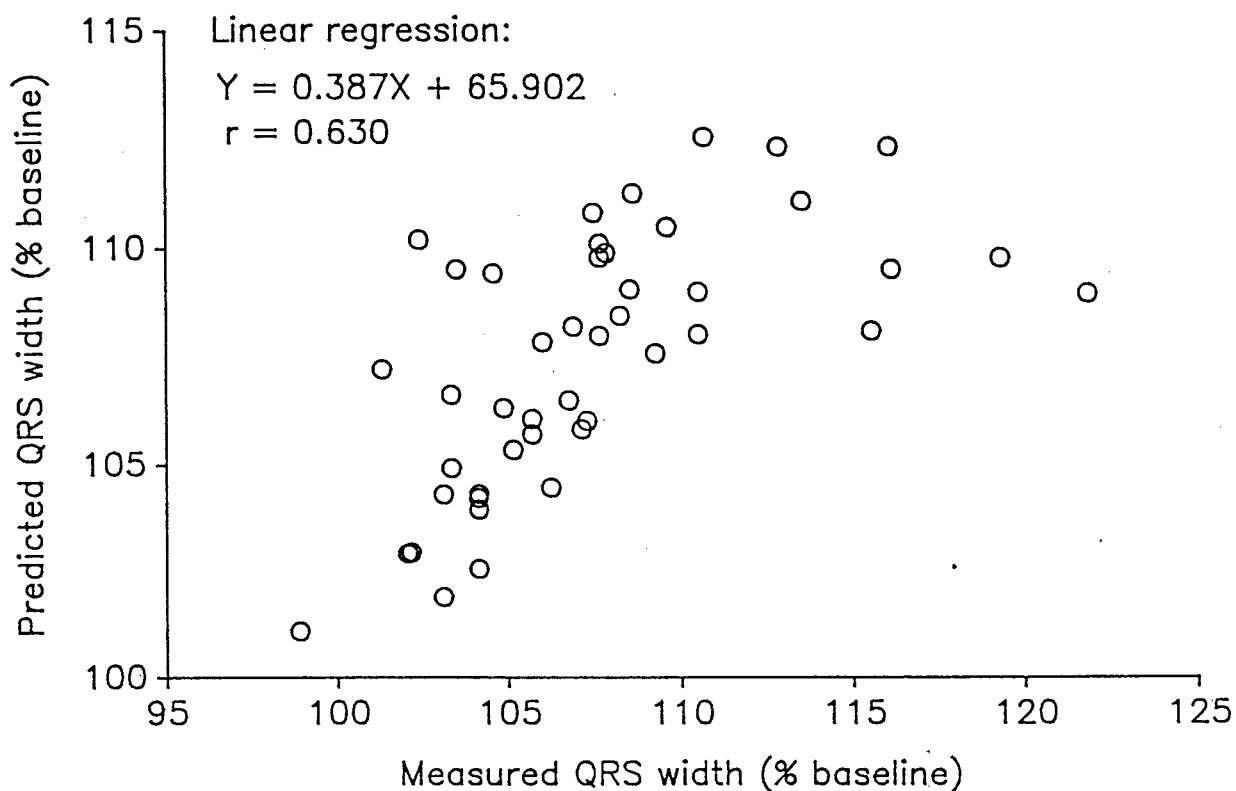


Figure 53. The correlation between measured QRS width and predicted QRS width. QRS width is predicted from the following equation (obtained by multiple stepwise regression): $Y = 0.004X_1' + 0.04X_2' + 379X_3 + 95$ where Y is QRS width, X_1' is PF serum concentration, X_2' is 5-hydroxy PF serum concentration.

4. DISCUSSION

4.1 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of Propafenone

The GLC-ECD assay method developed for the quantitation of PF demonstrated improved selectivity and sensitivity over the existing GLC method [Marchesini *et al.*, 1982] through the use of a bonded-phase fused-silica capillary column and the splitless injection technique. The limit of determination was ~2.5 ng/mL using 1 mL of serum. The method was further validated by comparison of its performance to a published HPLC method by Harapat and Kates [1982]. The two methods were shown to yield comparable quantitation of PF in serum. The GLC-ECD method demonstrated superior sensitivity to the published HPLC method (UV detection) with a lower limit of determination of 2.5 ng/mL using 1 mL of serum. On the other hand, the HPLC method can only be used to quantitate PF in samples (2-5 mL) with drug concentrations greater than 200 ng/mL (Table 10).

4.1.1. Splitless Injection and 'Cold Trapping' Effect

The sensitivity of the assay was greatly enhanced by the use of a splitless injection mode. The splitless mode of injection involves the introduction of a relatively large amount of sample into the capillary column without splitting off any sample to the vent [Freeman, 1981]. When using the splitless technique of sample injection, the solutes of the sample must be reconcentrated at the head of the column using either a 'solvent' or 'cold trapping' effect. With reconcentration, the band widths of the eluting peaks will reflect column efficiency rather than the volume

of the glass injection port liner [Freeman, 1981]. The basic difference between these two methods is the initial column temperature. The 'solvent' effect involves using an initial column temperature which is lower than the solvent boiling point [Grob and Grob, 1974]. On the other hand, the 'cold trapping' effect involves using an initial column temperature which is higher than the solvent boiling point but lower than the boiling point of the compound of interest [Freeman, 1981]. The 'cold trapping' effect was used effectively for PF (Figure 17).

4.1.2. Capillary Column

A bonded-phase fused-silica capillary column was employed for the analysis of PF. This type of column has the benefits of excellent thermal and chemical stability, efficiency, selectivity and sensitivity. The liquid phase of the column was 5% phenyl methylsilicone. Substitution of 5% of the methyl groups by phenyl moieties increases the polarity and selectivity of the liquid phase, as compared to the nonsubstituted methylsilicone.

4.1.3. Electron-Capture Detection and Acylation

The ECD is a highly selective and sensitive detector. Its high selectivity comes from its discrimination against non-electron capturing materials, allowing it to respond to only a few types of compounds (e.g. compound containing halogen atoms, anhydrides, aliphatic amines, esters, aldehydes and nitriles). Its high sensitivity offers the possibility of quantitative analysis at the nanogram or picogram level. The PF molecule contains a hydroxyl group and a secondary amine (Figure 1). Reaction of PF with fluorinated acid anhydrides such as TFAA or HFBA forms highly electron

withdrawing perfluoroacyl derivatives, making the compound more amenable to analysis by ECD. In addition to increased sensitivity and selectivity, derivatization also has the advantage of reducing peak tailing, which is common among compounds containing hydroxyl, carboxyl, amino and imino groups as a result of interaction with the GLC column [Ahuja, 1976]. The analysis time is also shortened due to the decreased polarity and increased volatility of the derivative. TFAA was the first agent used to derivatize PF. It was abandoned, however, due to interference from a negative peak. HFBA was then used as the acylating agent. Although ECD response is, in general, difficult to predict, there is evidence that detector response increases as the number of halogen atoms increases [Anggard and Hankey, 1969]. This was confirmed with the HFB derivative of PF where an approximate five-fold increase in sensitivity was observed as compared to the TFA derivative. The retention time was only minimally increased.

Acylation reactions can be performed in a non-polar solvent such as benzene or toluene with a tertiary amine as a catalyst. The tertiary amine serves as an acid acceptor, enhancing reactivity. Trimethylamine (TMA) in benzene is commonly used as a catalyst for the acylation of amino, alcoholic and phenolic compounds [Walle *et al.*, 1970; Ehrsson *et al.*, 1971; Walle *et al.*, 1971]. However, it has to be freshly prepared by saturation of benzene with gaseous TMA from TMA chloride and the TMA content determined titrimetrically [Walle *et al.*, 1971]. Because of the troublesome preparation and instability of TMA, TEA in toluene was used to replace TMA in benzene in our study. TEA is commercially available as a reagent and is chemically stable. We have demonstrated that by using TEA, the acylation time for PF reaction with HFBA was substantially shortened (Figure 7). Furthermore, TMA and TEA have the advantage over other basic

catalysts (such as pyridine) in that they do not cause disturbances in the gas chromatograms at high EC sensitivity settings. About 50 μL of 0.05 M of TMA has been recommended as a catalyst for acylation of amines [Walle *et al.*, 1971]. In the analysis of PF, a relatively smaller proportion of TEA (*i.e.* 400 μL of 0.003M TEA) was used to reduce solvent front band-spreading without affecting the acylation reaction (Figure 9). Acylation usually proceeds more slowly than other derivatization reactions and it is common to heat the samples to between 60°C and 100°C. Acylation of PF and I.S.-a carried out at 65°C appeared entirely satisfactory.

4.1.4. Extraction and Injection Solvent

Four solvents with different polarities were tested for their efficiency in extracting PF. In order of increasing polarity they were: hexane, toluene, benzene and toluene:dichloromethane:isopropyl alcohol (7:3:1). Benzene was chosen as the extraction solvent because its extraction efficiency was higher than that of the other solvents tested (Figure 11). Although the extraction efficiency of benzene ($E = 0.81$) was only slightly higher than that of toluene ($E = 0.76$), benzene extensively reduced (approximately by half) the time for sample evaporation during the analytical procedure. This was an important time-saving advantage, due to the large number of samples to be analyzed by this method during the pharmacokinetic studies of PF carried out during this project.

Solvents such as methylene chloride (dichloromethane), chloroform, carbon disulfide, diethyl ether, hexane and isooctane are widely used for splitless injection. Dichloromethane and chloroform cannot be used for ECD since these solvents have a high electron capture response. Because of the inertness of the bonded phase of modern fused silica capillary columns,

polar and aromatic solvents can also be used because they do not result in significant phase stripping due to the improved phase bonding technology in the manufacturing process of capillary columns [Freeman, 1981]. Although benzene was used as the extraction solvent, toluene was used as the derivatizing and injection solvent because of its higher boiling point. This has the advantage of considerably reducing the sample discrimination encountered in the splitless sampling mode due to the high injector port temperature and low boiling point of the solvent [Schomburg *et al.*, 1981].

4.1.5. Optimal GLC-ECD Conditions

The GLC-ECD conditions such as the inlet purge valve activation time, initial and final column temperature, temperature programming rate, injection port temperature, ECD temperature and make-up gas flow rate were all optimized to obtain the best peak resolution, band width and symmetry with the shortest retention time (Figure 13).

To reduce solvent tailing during splitless injection, the injection port was backflushed or purged some time after sample injection (*i.e.*, a temporary conversion of the sample inlet from a splitless mode back to a split configuration). This inlet purge valve activation time, which we determined as 40 seconds for PF (Figure 12), allowed most of the solute to pass through the column (*i.e.*, with a loss of $\leq 1\%$ of the solute) and removal of $\sim 5\%$ or less of the solvent, thereby reducing solvent tailing. Although there was no apparent difference in response between the various injection port temperatures tested (200-260°C) (Figure 13A), a lower temperature (210°C) was chosen. After sample injection in the splitless mode, the peaks of interest are actually broadened slightly in the injection inlet due to the low column flow rate and then recondensed into

narrow bands at the head of the column. At an initial low column temperature, the low boiling components proceed through the inlet to the column while the high boiling components are trapped at the head of the column until the oven temperature (*i.e.*, column) is increased. As a result, a lower injection port temperature is often more satisfactory in that it facilitates 'cold trapping' of high boiling solutes [Rooney, 1985].

The operation of an ECD requires a relatively high detector temperature to maintain detector cleanliness. Consequently, a detector temperature of 350°C was chosen to minimize contamination (Figure 13B). The ECD requires the use of a moderating gas to assure an equilibrium concentration of thermal electrons. This moderating gas can be used additionally as the sweep gas for the column exit [Freeman, 1981]. However, an optimum make-up gas flow rate has to be determined so that both sensitivity (favored by low flow rate) and band width (favored by high flow rate) can be obtained. Generally when using either hydrogen or helium as the carrier gas, a make-up flow rate of either nitrogen or argon/methane (95:5) at 20-60 mL/min appears to be satisfactory [Freeman, 1981]. In the assay development for PF, argon/methane (95:5) was used as the make-up gas and a flow rate of 60 mL/min seemed to give the optimum sensitivity and the minimum band width for PF (Figure 13C).

4.2 Electron-Capture Detection Capillary Gas-Liquid Chromatographic Analysis of 5-Hydroxy Propafenone and 5-hydroxy-4-methoxy Propafenone

Although it is desirable to measure the intact drug and its metabolite(s) simultaneously, occasionally separate assay methods are

necessary because of the different physico-chemical nature of the metabolite(s). The metabolite, 5-hydroxy PF, has increased acidity and polarity compared to the parent drug due to the hydroxyl substituent on the benzene ring (Figure 2). Therefore, simultaneous measurement of both compounds was not possible with the present method without significant sacrifice of the recovery of PF or its primary metabolite. The GLC-ECD method developed for the quantitation of PF was, therefore, further modified for the measurement of 5-hydroxy PF.

The more polar solvent mixture - toluene:dichloromethane:isopropyl alcohol (7:3:1) had the highest extraction efficiency ($E = 0.81$) of the four solvents tested and was used to replace benzene ($E = 0.76$) as the extraction solvent for 5-hydroxy PF (Figure 20). The phenolic group on 5-hydroxy PF is acidic and can form a water-soluble phenolate salt with the strong alkali, sodium hydroxide. Therefore, sodium and potassium carbonate were used to replace sodium hydroxide during the extraction to minimize the formation of such water-soluble sodium salt of 5-hydroxy PF. In addition to the hydroxyl group and the secondary amine, the phenolic group of 5-hydroxy PF is also acylated by HFBA. Temperature programming and other GLC-ECD conditions were optimized to obtain complete separation of 5-hydroxy PF from PF, I.S.-b and endogenous components of the serum.

4.3 Structural Confirmation of the HFB Derivatives of Propafenone and 5-Hydroxy Propafenone

The EI-MS fragmentation of the HFB derivative of PF in the present study exhibited a pattern similar to the MS fragmentation of a TFA

derivative of PF reported by Hege *et al.* [1984a]. The ion at m/e 733 was the molecular ion (M^+), consistent with the reaction of PF with two molecules of HFBA (Figure 25). As compared to EI-MS, the PICI-MS fragmentation of the HFB derivative of PF had fewer fragment ions. In addition to the pronounced $(M+1)^+$ ion, characteristic ions of $(M + C_2H_5)^+$ and $(M + C_3H_5)^+$ were also found when methane was used as an ionizing gas (Figure 27). The fragment ions at m/e 508, 294 and 252 appeared in both EI-MS and PICI-MS profiles.

The NICI-MS fragmentation of the HFB derivative of PF in the present study followed a pattern similar to that of the derivatives of metoprolol and oxprenolol formed by HFB and reported by Gaudry *et al.* [1985a,b]. Structurally, metoprolol and oxprenolol have side-chains that have the same molecular formulae as PF ($C_3H_7-NH-CH_2-CH(OH)-CH_2-$, $+2HFB = 508$), and the fragment ions at m/e 488 ($508 - HF$) and 448 ($508 - 3HF$) were the common ions in the NICI mass spectra of these three compounds. Other common fragment ions observed were at m/e 213 ($C_3F_7COO^-$) and 194 ($213 - F$). The $(M-1)^-$ ion (m/e 732) was characteristic of NICI-MS. The molecular ion lost a progressively increasing number of HF fragments to yield ions at m/e 693 ($733 - 2HF$), 673 ($733 - 3HF$) and 653 ($733 - 4HF$) (Figure 29).

The EI-MS, PICI-MS and NICI-MS of I.S.-a follow a similar pattern as PF.

The EI-MS of 5-hydroxy PF showed a similar pattern to the EI-MS of PF. The common ions include those at m/e 508, 91, 43, 294, 252, 104, 226, 466 and 77. The molecular ion (M^+) at m/e 945 was not observed in the mass spectrum (Figure 33). The ions at m/e 333, 359 and 748 are characteristic of the HFB derivative of 5-hydroxy PF since it indicates the acylation of the phenolic functional group of 5-hydroxy PF, in addition to the

derivatization of the hydroxyl and secondary amine.

4.4 *In Vitro* Serum Protein Binding Study of Propafenone

4.4.1 Protein Binding Technique

Several methods including equilibrium dialysis, ultrafiltration, ultracentrifugation, gel filtration, etc. have been used for the determination of drug protein binding or free fraction [review in Kurz *et al.*, 1977; Wandell and Wilcox-Thole, 1983; Barre *et al.*, 1988]. Among these techniques, equilibrium dialysis and ultrafiltration are the most commonly used methods in the laboratory setting [Kurz *et al.*, 1977; Bowers *et al.*, 1984; Kwong, 1985]. Equilibrium dialysis is often regarded as the reference method and has the advantage of free component retaining access to the bound component when equilibrium is achieved [Briggs *et al.*, 1983]. Ultrafiltration is less reliable but also less time-consuming than equilibrium dialysis. Although ultrafiltration has the advantage of speed and ease, the extensive adsorption (16.2%) of PF to the ultrafiltration device makes this technique unacceptable for PF protein binding studies, since it leads to an underestimation of free drug concentration or free fraction. The non-specific binding of PF to the equilibrium dialysis membrane (2.3%) is low. Although the adsorption of PF to the equilibrium dialysis cell is similar to that of ultrafiltration device (16.6%) (Table 11), it does not affect the calculation of free fraction because both the buffer and the serum were assayed for PF concentrations after equilibrium dialysis allowing for the necessary correction [Nimmo *et al.*, 1977; Bowers *et al.*, 1984]. The sensitivity of the GLC-ECD method developed for PF is

entirely adequate for direct free drug measurement in our *in vitro* protein binding study, the single-dose enzyme induction study and the concentration-response relationship study where, in all instances, low sample volumes were encountered. Although using radiolabelled drug in equilibrium dialysis will further improve the assay sensitivity, experimental accuracy depends on the purity [Builder and Segel, 1978] and the stability of the radiolabelled drug. Furthermore, the radiolabelled substances in the dialysate after dialysis have to be identified in order to obtain valid data on the binding of highly bound radiolabelled compounds [Yacobi and Levy, 1975].

Besides non-specific adsorption of drugs to dialysis membranes and apparatus [Briggs *et al.*, 1983], other problems associated with the use of equilibrium dialysis for protein binding study include volume shift [Lima *et al.*, 1983; Tozer *et al.*, 1983; Boudinot and Jusko, 1984], pH shift [Brinkschulte and Breyer-Pfaff, 1979; Lui and Chiou, 1986], and, as well, bacterial growth [Ilett *et al.*, 1975; Brinkschulte and Breyer-Pfaff, 1979; Briggs *et al.*, 1983].

Volume shift is the net movement of water from the buffer chamber into the serum chamber, probably due to the osmotic effect of plasma proteins, resulting in a reduction in the concentration of the binding proteins. It is time-dependent and has been shown to have a significant effect on binding if the dialysis time exceeds ~12 h [Lima *et al.*, 1983]. Volume shift can be corrected by use of derived equations [Lima *et al.*, 1983; Tozer *et al.*, 1983; Boudinot and Jusko, 1984]. It can be attenuated by the inclusion of dextran (M.W. 70,000, 55% of the total protein concentration) in the buffer to decrease the osmotic pressure gradient existing between the protein and buffer sides of the membrane. It can also

be attenuated by use of a thick, low M.W. cutoff membrane during equilibrium dialysis [Lima *et al.*, 1983]. Lima *et al.* studied the consequence of volume shift on the binding of clofibrate, lidocaine, disopyramide, propranolol and diazepam and found that drugs with higher plasma protein binding seemed to have lower volume shift. In our protein binding study, we did not observe any apparent volume shift, probably due to the short dialysis time (6 h) and the high protein binding of PF.

Lui and Chiou [1986] have shown that there was no significant change in pH in the dialysis solutions (phosphate buffer, pH 7.4) if the dialysis time was less than 6 h, although they recommended presoaking of the dialysis cells in 70% ethanol overnight to minimize variability in the pH of the dialysis solutions. When longer dialysis times are necessary, bacterial growth can cause a pH shift in dialysis solutions and/or a decrease in the amount of protein available for drug binding as a result of hydrolyzation of plasma proteins. Bacterial growth has been shown to reduce the percent of protein binding of quinidine in dog plasma, and salicylate in human plasma [Ilett *et al.*, 1975]. The use of kanamycin to inhibit bacterial growth seemed to solve the problem without interfering with the protein binding of the drugs tested [Ilett *et al.*, 1975]. Another solution was the use of sodium azide as a preservative to prevent pH shift but caution must be exercised if azide ions interfere with the binding of the drug of interest [Lui and Chiou, 1986].

4.4.2 Binding Characteristics and Concentration-Dependent Serum Protein Binding of Propafenone

Two main classes of binding sites on serum proteins were identified for PF, one with high-affinity and low-capacity and the other with low-

affinity and high-capacity (Figure 36). The high-affinity, low-capacity binding site is usually associated with AAG while the low-affinity, high-capacity binding site is generally associated with albumin [Javaid *et al.*, 1983]. We have also demonstrated that the serum protein binding of PF is concentration-independent *in vitro* within the concentration range of 0.25-1.5 $\mu\text{g/mL}$. However, the binding is concentration-dependent at PF concentrations greater than 1.5 $\mu\text{g/mL}$ (Table 12). Using purified human AAG, Gillis *et al.* [1985] showed that PF was bound to this protein and there were two classes of binding sites on AAG for PF, both with high affinity. Serum AAG concentration (normal range: 70-110 mg/dL) is normally 100 times lower than serum albumin concentration (normal range: 3.9-5.5 g/dL). Furthermore, AAG represents a high-affinity, low-capacity binding site which can be readily saturated by increasing drug concentrations [Routledge, 1986]. It may explain why PF exhibited apparent concentration-dependent serum protein binding at higher PF concentrations.

4.4.3 Clinical Implication of the Concentration-Dependent Serum Protein Binding of Propafenone

PF has been demonstrated to have potential for use as an effective antiarrhythmic agent [Rudolph *et al.*, 1979; Connolly *et al.*, 1983a; Breithardt *et al.*, 1984; Hodges *et al.*, 1984; Podrid *et al.*, 1984; Salerno *et al.*, 1984]. However, such treatment is sometimes accompanied by severe side effects, most notably cardiovascular in origin [Schlepper, 1987]. The therapeutic range of PF is suggested to be 0.5-2 $\mu\text{g/mL}$ [Seipel and Breithardt, 1980], although a great interindividual variability has been shown [Connolly *et al.*, 1983b; Salerno *et al.*, 1984; Siddoway *et al.*, 1984a] and considerable overlap between the therapeutic and toxic ranges

has been demonstrated. In the presence of concentration-dependent binding, the free drug concentration is no longer a constant fraction of the total plasma concentration and a slight change in total drug concentration may cause a fluctuation in free drug concentration leading to an alteration in the pharmacological effect (therapeutic and/or toxic). No evidence for significant concentration-dependent changes in free fraction was observed within the PF concentration range of 0.5-1.5 $\mu\text{g/mL}$, which covers the greatest proportion of the proposed therapeutic range (0.5-2 $\mu\text{g/mL}$). However, concentration-dependent binding was demonstrated at PF concentrations greater than 1.5 $\mu\text{g/mL}$. In other words, fluctuations of free drug concentration may occur when PF serum concentrations approach the upper end of the proposed therapeutic range. When there is an untoward accumulation of PF in patients, for example, as seen with hepatic dysfunction [Lee et al., 1987], it would appear that the potential exists for variable PF free fraction at the upper limits of clinically encountered therapeutic drug concentrations. Alternatively, fluctuations in free PF concentration could also be affected by factors such as age, concomitant drug therapy (displacement of PF from protein binding site) and certain physiological or pathological states which can cause a qualitative or quantitative change in the binding protein(s) or alterations in the distribution of the binding protein(s) in the body [Wandell and Wilcox-Thole, 1983; Levy and Moreland, 1984; Kwong, 1985].

4.4.4 Effect of Uremia and Renal Failure on the Serum Protein Binding of Propafenone

The effect of renal disease on plasma binding of basic drugs is heterogeneous. The binding may be increased, unchanged or decreased,

depending on the relative contribution of the individual plasma proteins to the total binding and the type and severity of disease [Piafsky, 1978; Piafsky, 1980]. Patients with terminal uremia have been shown to have increased concentrations of AAG in plasma [Henriksen *et al.*, 1982; Docci and Turci, 1983; Pacifici *et al.*, 1986]. In our study, the AAG concentrations observed in mid-range uremic patients were not significantly different from those observed in healthy subjects. However, the AAG concentrations in patients with renal failure were twice that noted in healthy volunteers and propafenone free fraction was reduced by approximately 30% (Figure 38). With the exclusion of the data of one renal failure patient, who had a much lower AAG concentration (98.8 mg/dL) compared to the other patients and a PF free fraction (0.042) which was not significantly different from healthy volunteers, the free fraction of PF was reduced by approximately 44% in serum from patients with renal failure. In pooled uremic serum, the free fraction of PF was approximately 50% of that observed in normal serum throughout the concentration range studied (1-5 $\mu\text{g/mL}$) (Table 12). The decreased PF free fraction in patients with increased serum AAG concentration, as well as the positive and significant correlation between AAG concentration and the binding ratio of PF observed further implies that AAG is an important binding protein for PF in serum, as noted by Gillis *et al.* [1985]. This is in accordance with the finding that protein binding of some basic drugs, such as diazepam and lidocaine, which bind largely to AAG, was increased in plasma obtained from patients with renal failure and the increased binding were associated with increased AAG concentration [Grossman *et al.*, 1982]. Similar results were also demonstrated between increased AAG concentrations and decreased drug free fraction for lidocaine [Edwards *et al.*, 1981] and quinidine [Edwards *et*

et al., 1984] in plasma obtained from patients after traumatic injury. In a variety of inflammatory conditions and other disease states when AAG concentration may increase four or five-fold [Wilkinson, 1983], a potential increase in protein binding can be expected. The plasma protein binding of propafenone may also increase in patients with cardiac dysrhythmias complicated by myocardial infarction, since an increase in AAG concentration after myocardial infarction is well documented [Johansson *et al.*, 1972; Snyder *et al.*, 1975], and thus free drug monitoring may be appropriate in these patients.

4.4.5 Free Drug Monitoring of Propafenone

PF is a high clearance drug and its hepatic elimination is perfusion rate-limited. That is, its clearance depends on hepatic blood flow and is not affected by plasma protein binding changes [Wilkinson and Shand, 1975]. As a result, average steady-state serum total drug concentration is unaffected by changes in protein binding. However, average steady-state serum free drug concentration will be affected [MacKichan, 1984; Rowland, 1984]. Since total drug concentration is more commonly monitored than free drug concentration, a change in free drug concentration may go undetected. In those instances where a drug is highly protein bound, possesses a narrow therapeutic range, displays a poor dose-effect relationship and exhibits a concentration-dependent binding, the measurement of free drug concentration should be considered to improve therapeutic response and reduce toxicity [Levy and Moreland, 1984]. Since PF displays most of the above mentioned characteristics, it would appear that this drug may benefit from the assessment of free drug concentrations in serum.

4.5 Phenobarbital Treatment in Healthy Non-Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

4.5.1 Data Fitting

When the serum concentration *versus* time data of PF was first fitted into AUTOAN, the kinetic parameters for the best fit pharmacokinetic open-model were obtained. These initial parameters were then analyzed by NONLIN [Metzler, 1974] to obtain a better estimate of β , k_a and other parameters. However, in some subjects, appreciable error was noted in the apparent best fit processed by NONLIN as compared to AUTOAN or hand stripping of the data. Since the value of β estimated from AUTOAN were not significantly different from values obtained by hand stripping of the data, the estimates from AUTOAN were used. In addition, the calculation of most of the kinetic parameters was not affected by model fitting since C_{max} and t_{max} were obtained from individual serum concentration *versus* time curves while AUC and V_{dss} were obtained by non-compartmental methods.

4.5.2 Statistical Analysis

In the analysis of the difference in the kinetic parameters before and after phenobarbital treatment, the Wilcoxon paired-sample test was used. It is a nonparametric analogue of or alternative to the paired t-test (Zar, 1984). Since we can not assume that the differences in the kinetic parameters before and after phenobarbital treatment are normally distributed, the Wilcoxon paired-sample test is more appropriate.

4.5.3 Interindividual Variation in Kinetic Parameters of Propafenone

There was a large interindividual variation in most of the

pharmacokinetic parameters of PF between non-smoking subjects (Table 13). Factors that have been shown to contribute to the variation in drug disposition [Vesell, 1982; Sellers *et al.*, 1983] were identified or carefully controlled in our study. These factors include age (selection of subjects was restricted to a population age range of 20-45 y), body composition (normal weight and normal height), racial background (Caucasian), sex (male), tobacco exposure (non-smokers who did not have a history of smoking or who had not smoked a cigarette for 15 years), alcohol consumption (mild to moderate) and disease state (healthy). In addition, subjects fasted for 12 h prior to the study and did not ingest any food until 4 h after PF administration and the contents of the meals (lunch and dinner) on the first day of each phase of the study were controlled (all subjects were given the same type of food) to decrease the influence of this factor on PF metabolism, since the bioavailability of PF was shown to be significantly influenced by food [Axelson *et al.*, 1987]. However, the long-term effect of diet, which has been shown to contribute to interindividual variations in drug metabolism [Vesell, 1984], was not considered in our study. Furthermore, although all non-smoking subjects were 'rapid' metabolizers based on the value of their CL_{int} control (≥ 0.5 L/min), there was a wide range in this parameter among the subjects (0.8-13.3 L/min), indicating a large interindividual variation in their ability to metabolize drugs. Therefore, physiological factors (e.g. nutritional status), long-term effect of diet and genetic factor (the individual's ability to metabolize drugs), as well as other environmental factors (e.g. environmental or occupational exposure to drugs and other xenobiotics) [Conney *et al.*, 1977; Mucklow, 1988] may have contributed to the variation in the pharmacokinetic parameters of PF observed in these

subjects.

4.5.4 Induction of Propafenone Metabolism by Phenobarbital

Intrinsic clearance indicates the maximal ability of the liver to irreversibly remove drug by all pathways in the absence of any flow limitations [Wilkinson and Shand, 1975]. Phenobarbital induces hepatic metabolizing enzyme activity and increases the CL_{int} of PF. The increase in CL_{int} of PF after phenobarbital treatment was highly variable among subjects, ranging from a 1.1 to a 9.3 fold increase in this parameter (Table 13). The effect of increasing intrinsic clearance on the total blood concentration *versus* time curve after oral administration of a totally metabolized high clearance drug was fully described and discussed by Wilkinson and Shand [1975]. For a high clearance drug like PF, induction can only increase the extraction ratio by a small factor since its initial extraction ratio is already very high (close to one). As a result, there was minimal change in hepatic clearance and therefore no change in the $t_{1/2\beta}$ of PF. However, this change increased even further the already extensive first-pass metabolism after oral administration and decreased the systemic availability of PF. This was observed in the significant reduction in C_{max} and AUC of PF after phenobarbital treatment (Table 13). The percent decrease in C_{max} after enzyme induction was very similar to the percent decrease in serum AUC in most subjects.

The oral clearance of several β -adrenergic blockers, including metoprolol, alprenolol and propranolol, has been shown to be increased by 50-500% after rifampicin and phenobarbital treatment [Branch and Herman, 1984]. The oral clearance of PF was increased by 10-831% in the non-smoking subjects after phenobarbital treatment.

Since PF undergoes aromatic hydroxylation to 5-hydroxy PF, a pathway known to be induced by phenobarbital, it was anticipated that an increase in the serum concentration of 5-hydroxy PF after enzyme induction would be observed. Contrary to what was expected, the serum levels, C_{\max} and AUC of 5-hydroxy PF were reduced after phenobarbital treatment (Table 16). Since 5-hydroxy PF is further metabolized to glucuronide and sulfate conjugates, an increase in the serum level of 5-hydroxy PF may not be observed if aromatic hydroxylation is still the rate-limiting step after enzyme induction.

Eichelbaum *et al.* [1986] studied the effects of administration of three enzyme inducing agents, antipyrine, phenobarbitone and rifampicin, on sparteine metabolism in 'extensive' and 'poor' metabolizer subjects. They found that sparteine metabolism was induced in both 'extensive' and 'poor' metabolizer subjects. However, the oxidation pathway was not induced since there was a decrease in the urinary accumulation of 2 and 5-dehydro-sparteine. Eichelbaum *et al.* suggested that 2 and 5-dehydrosparteine undergo further metabolism and that this metabolic pathway was induced. They also found that in 'poor' metabolizer subjects, enzyme induction did not lead to a change in phenotype. They concluded that the regulation of the cytochrome P-450 isozyme involved in polymorphic debrisoquine/sparteine metabolism is predominantly under genetic control [Inaba *et al.*, 1980; Eichelbaum *et al.*, 1982; Inaba *et al.*, 1982] and that enzyme inducing agents have a minor influence [Eichelbaum *et al.*, 1986]. Propafenone undergoes metabolism *via* a polymorphic oxidative pathway, presumably by the same isozyme as for debrisoquine and sparteine [Siddoway *et al.*, 1983; Siddoway *et al.*, 1987]. It is possible that the cytochrome P-450 isozyme involved in polymorphic oxidative metabolism of PF is non-inducible.

Glucuronidation is a pathway known to be induced by phenobarbital. A reduction in the renal excretion of the conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF (expressed as percent of dose) was also noted (Table 19). This can be explained by the fact that the major excretion route for the glucuronides of 5-hydroxy PF in man is biliary excretion followed by faecal excretion without appreciable enterohepatic circulation [Hege *et al.*, 1984a]. Furthermore, chronic administration of phenobarbital and other enzyme inducing agents increases the biliary excretion of certain xenobiotics and/or metabolites [Fujimoto *et al.*, 1965; Bernstein *et al.*, 1968; Goldstein and Taurog, 1968; Klaassen, 1970; Levine, 1970a, 1970b; Schlede *et al.*, 1970; Levine, 1972; Cooke *et al.*, 1973; Javor *et al.*, 1973; Levine, 1974; Whelan and Combes, 1975; Roerig *et al.*, 1976]. Four mechanisms for this process have been proposed [Whelan and Combes, 1975]. First, phenobarbital increases the liver content of a cytoplasmic organic anion binding protein, ligandin [Reyes *et al.*, 1971], thereby increasing the hepatic uptake of drug or metabolite. This will increase the biliary excretion of a compound only if hepatic uptake is the rate-limiting step in hepatic transport from blood to bile. Second, phenobarbital induces the microsomal drug-metabolizing enzymes and increases the hepatic metabolism of drugs [Conney, 1967]. This will increase the biliary excretion of a metabolite if the metabolite is excreted into the bile at a rate faster than that of the intact drug. Third, phenobarbital increases biliary flow [Klaassen, 1969] and increases the efficiency of transport of drug or metabolite from liver cells into bile. Fourth, phenobarbital increases liver size [Conney, 1967] and hepatomegaly includes not only hypertrophy but also hyperplasia of hepatocytes [Fouts and Rogers, 1965; Argyris, 1968]. Phenobarbital treatment may result in the synthesis of some new

canalicular transporting units which facilitate the transport of a compound into the bile [Whelan and Combes, 1975]. This possibility is supported by the fact that increased liver size and increased biliary flow are characteristics of phenobarbital treatment, which are not shared by other enzyme inducing agents such as 3-methylcholanthrene and 3,4-benzo(a)pyrene [Klaassen, 1969]. For drugs that are metabolized in the liver prior to biliary excretion, it is difficult to identify the true mechanism responsible for enhanced biliary excretion since the overall process includes hepatic uptake, hepatic metabolism and transport from liver cells into bile. This is further complicated by the fact that the rate-limiting step for the overall process can be different for different compounds. For example, metabolism is the rate-limiting step for compounds such as methadone [Roerig *et al.*, 1976], 3-methylcholanthrene [Levine, 1972], 3,4-benzo(a)pyrene [Levine, 1970a] and 7,12-dimethylbenzanthracene [Levine, 1974] while transport into the bile is the rate-limiting step for compounds such as phenolphthalein, 4-methyl-umbelliferone and 8-hydroxyquinoline [Mulder, 1973]. It was suggested that when phenobarbital enhances the biliary excretion of a compound, more than one of the above mechanisms may be stimulated. If phenobarbital does increase the biliary excretion of the glucuronides of 5-hydroxy PF and since biliary excretion is the major excretion route for this metabolite, an increase in the amount of glucuronides as a result of enzyme induction may be observed in the feces and not in the urine. This would then explain the lack of an increase in the proportion of dose being excreted by this pathway.

Collste *et al.* [1979] examined the influence of pentobarbital on the effect and plasma levels of alprenolol and its metabolite, 4-hydroxy alprenolol. They found that ten days of pentobarbital treatment decreased

the plasma concentrations of both alprenolol and 4-hydroxy alprenolol. The authors concluded that conjugation of 4-hydroxy alprenolol or other metabolic pathways were induced. The fact that the induction of PF metabolism by phenobarbital did not result in an increase in either the plasma concentrations of 5-hydroxy PF or the urinary excretion of the conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF may be due to an induction of metabolic pathways other than aromatic hydroxylation and glucuronidation. These other metabolic pathways may become important after phenobarbital treatment. Indeed, an induction of the 'inducible' pathways can cause a reduction in the 'non-inducible' pathways, which may explain why we observed a decrease in the serum levels of 5-hydroxy PF after enzyme induction.

4.5.5 Salivary Excretion of Propafenone

The excretion of drugs in human saliva was reported as early as in 1965 by Borzelleca and Cherrick. Since then, the salivary excretion of a wide variety of drugs including anticonvulsants (e.g. barbiturates, carbamazepine, phenytoin), salicylates, sulfonamides, antipyrine, theophylline, lithium, antiarrhythmic agents (e.g. procainamide, quinidine, disopyramide, lidocaine), psychotropic agents (e.g. chlorpromazine, diazepam) and ethanol have been examined and investigated by different researchers [review in Mucklow, 1982]. It is generally accepted that the unbound (free) drug in plasma was in equilibrium with drug in saliva. Therefore, most of the previous studies focused on the correlation between saliva and plasma drug levels and factors that affected salivary drug level. For a number of compounds a close, linear correlation has been established between plasma and saliva levels. Measurement of salivary drug

concentration has the advantage of being a simple, easily accessible and painless (non-invasive) way of obtaining pharmacokinetic data (e.g. antipyrine, salicylate) [Graham and Rowland, 1972; Fraser et al., 1976], estimating drug plasma protein binding (e.g. amobarbital) [Inaba and Kalow, 1975], assuring compliance and monitoring drug therapy (e.g. phenytoin, carbamazepine) [Rylance and Moreland, 1981] and establishing the existence of metabolic induction by phenobarbital of the antiarrhythmic agent, disopyramide [Kapil, 1985; Kapil et al., 1987].

Killmann and Thaysen [1955] demonstrated that the relationship between saliva and plasma concentrations of different sulphonamides was dependent on the pK_a of the drug and the pH of both saliva and plasma. Mucklow and co-workers [1978] have further demonstrated that for weak acids with $pK_a > 7$ [e.g. phenobarbital (pK_a 7.2), phenytoin (pK_a 8.3)] and weak bases with $pK_a < 5.5$ [e.g. antipyrine (pK_a 1.4)], the saliva/plasma drug concentration ratio is constant, irrespective of any change in saliva flow rate or pH. However, for acidic drugs with $pK_a < 7$ [e.g. chlorpropamide (pK_a 4.8), tolbutamide (pK_a 5.4)] and basic drugs with $pK_a > 5.5$ (e.g. meperidine (pK_a 8.7), propranolol (pK_a 9.4)], the ratio is extremely sensitive to any change in saliva flow rate or pH. Since PF is a weak base with a pK_a of 9.0 and is largely ionized at serum pH (7.4), it could be anticipated that its partitioning into saliva (pH 5.5-7.8) may be high and dependent on salivary pH. This is apparently true as shown by the negative and significant (control, $r = -0.461$; phenobarbital-treated, $r = -0.422$; $p < 0.05$) correlation found between the saliva/serum PF concentration ratio and the pH of saliva. This indicates that an increase in the pH of saliva will cause a decrease in salivary PF concentration. Our results also showed that PF salivary concentration in the non-smokers was considerably

higher than PF serum free concentration (Table 17). Propafenone salivary concentration was approximately 21% (control) and 27% (phenobarbital-treated) of PF serum total concentration, with considerable inter- and intraindividual variation (Table 15). The mean saliva/serum PF concentration ratio of the eight non-smokers in the control state was 0.21 ± 0.08 . This is similar to the result obtained by Mason *et al.* [1987], who found that the saliva/plasma PF concentration ratio at steady-state (300 mg PF every 8 h for 6 days) in 24 healthy male subjects was 0.25 ± 0.11 .

Matin *et al.* [1974] studied the salivary excretion of tolbutamide. They attempted to correlate saliva and plasma drug concentrations with the ultimate goal of predicting plasma drug levels from salivary excretion data. Inaba and Kalow [1975] used the equation developed by Matin *et al.* [1974], which incorporates saliva/serum drug concentration ratio, pK_a of the drug and pH of saliva and plasma, to calculate the percent of unbound (free) amobarbital in serum and found that the estimated and measured (by equilibrium dialysis) values were in good agreement. A similar equation developed for basic drugs [Equation 7, Matin *et al.*, 1974] was used to calculate the percent of free (unbound) PF in serum. If PF concentration in saliva is truly an indicator of PF free concentration in serum, then by measuring PF serum total concentration, PF salivary concentration and saliva pH, an estimation of PF free fraction can be obtained without the more time-consuming method of equilibrium dialysis. Our results showed that the estimated percent of free PF in serum was significantly higher than the values obtained from equilibrium dialysis (Table 17), indicating that other factors may affect or be involved in the excretion of PF in saliva. The discrepancy can perhaps be explained by the hypothesis and assumption of which Equation 7 was based on. These include: a) excretion

is a simple passive diffusion process; b) only the non-protein bound and the unionized form of the drug can diffuse across the epithelium of the salivary gland [Killmann and Thaysen, 1955]; c) the unbound drug in serum is in equilibrium with saliva [Huffman, 1975; Koysooko *et al.*, 1974]; d) equilibrium across the membrane is reached in spite of the continuous secretion and outflow of saliva and e) drug is unbound in saliva or protein binding of drug in saliva is negligible [Killmann and Thaysen, 1955; Rasmussen, 1964; Martin *et al.*, 1974]. Some of the above assumptions may not be true for PF.

Borzelleca and Putney [1970] have demonstrated, in the dog, that salivary excretion of salicylate is more than simple diffusion of the drug across membranes. The salivary excretion of salicylate is best described by two processes including filtration of drug (including ionized form) across the basal membrane through aqueous pores into the epithelial cell, followed by diffusion of the drug (only unionized form) across the apical membrane into the salivary fluid. The first process is governed by a concentration gradient and is independent of pH while the second process depends on saliva pH and is rate-limiting. The possibility of active transport cannot be ruled out, although only limited evidence is available to suggest active secretion of drugs (so far only lithium, penicillin, metoprolol and tocainide) into saliva [Borzelleca and Cherrick, 1965; Groth *et al.*, 1974; Dawes *et al.*, 1978; Pillai *et al.*, 1984]. In the case of tocainide, stereospecific salivary excretion of tocainide enantiomers [R(-) and S(+) isomers] has been observed. The transfer of tocainide enantiomers from plasma to saliva does not correlate with salivary pH and is more pronounced for the R(-)-enantiomer of the drug [Pillai *et al.*, 1984].

There are two potential sources of error that may cause incorrect

estimation of percent of free drug in serum by Equation 7 [Mucklow, 1982]. First, the pH of saliva measured after expectoration may be higher than the pH of saliva at the moment of secretion, due to the loss of carbon dioxide, formed from carbonic acid in solution, when saliva comes into contact with air. Second, drug may bind to particulate matter in whole saliva (e.g. phenytoin) [Anavekar *et al.*, 1978] or to buccal mucosa (e.g. trimethoprim) [Eatman *et al.*, 1977]. However, they are unlikely to cause any error in the estimation of percent free drug for PF using Equation 7, since both should give falsely low estimates of percent of free drug in serum for a basic drug [Mucklow, 1982].

Another source of error is perhaps the contamination of saliva samples when the drug tested is administered orally [Paxton and Foote, 1979]. However, it should not be a major problem unless the drug was taken as a syrup, chewable tablets or crushed tablets (to children). In addition, contamination should only affect saliva samples collected shortly after administration of the drug. In our study, contamination was unlikely since the subjects drank 200 mL of water after the tablet was swallowed. Furthermore, aberrantly high PF levels were not observed in saliva samples collected shortly after PF administration, indicating that there was no contamination during the process of ingestion.

It has been demonstrated that many factors can affect salivary excretion of drugs. In addition to pK_a (degree of ionization), the lipid solubility of the drug (reflected by the solvent/water partition coefficient), molecular weight and spatial configuration may also determine the diffusibility of the compound across the membrane [Amberson and Hober, 1932; Rasmussen, 1964]. Saliva flow rate is another important factor that can affect diffusion of the drug and attainment of equilibrium across the

epithelial membrane of the salivary glands. Since PF is ionized to an appreciable extent at serum pH, it would seem that saliva pH and flow rate, rather than serum pH, would have a greater effect on the salivary excretion of PF. The flow rate of whole saliva in man varies from 0.5-2.1 mL/min, with considerable individual variation [Becks and Wainwright, 1943]. Stimulation, age [Gutman and Ben-Aryeh, 1974], anxiety and personality (conscientiousness, shrewdness and introversion) [Costa *et al.*, 1980] can affect the flow rate of saliva and are responsible for the variability in both saliva flow rate and saliva pH, since the latter varies in direct proportion to saliva flow rate [Mucklow *et al.*, 1978]. The pH of stimulated saliva has been demonstrated to rise by as much as two units, depending on the increase in flow rate [Dawes and Jenkins, 1964]. The salivary concentration *versus* time curves of PF (Appendix 3) showed that salivary concentrations of PF in some subjects fluctuated over the 48 h period. Since mechanically unstimulated whole saliva was collected in our study, the fluctuations in salivary PF concentrations observed in some subjects may be due to the circadian rhythm of saliva flow rate. The reproducibility in the individual and the considerable interindividual variation in both the circadian and circannual rhythms of saliva flow rate and composition have been fully and extensively studied [Dawes, 1972; Dawes and Ong, 1973; Ferguson *et al.*, 1973; Ferguson and Fort, 1974]. However, whether fluctuations in salivary PF concentration follow the same pattern as the circadian rhythm of saliva flow rate in the individual subject has yet to be established.

The correlation between salivary PF concentration and serum PF free concentration was significant ($p < 0.05$) but not close ($r = 0.435$). Furthermore, there was a better correlation between serum total

concentration and serum free concentration ($r = 0.838$, $p < 0.001$) than that between serum total concentration and salivary concentration ($r = 0.702$, $p < 0.001$) and that between salivary concentration and serum free concentration ($r = 0.435$, $p < 0.02$) (Figure 41). Mason *et al.* [1987] also found a significant but not close correlation between steady-state PF serum concentration and steady-state PF salivary concentration. This suggests that PF salivary concentration is not a particularly good indicator of PF serum free concentration and, therefore, should be used with caution when predicting the corresponding serum drug level.

One interesting observation was that the decrease in salivary AUC was very similar to, and not statistically significant from, the decrease in serum AUC after phenobarbital treatment. In situations when serial blood sampling is not possible, saliva sampling may provide an alternative way of estimating alterations in serum AUC. This observation with PF seems even more predictive than that seen when a similar study was conducted in our laboratory with disopyramide, where the variability of salivary levels was greater than that seen in the present study [Kapil, 1985; Kapil *et al.*, 1987].

4.5.6 Protein Binding of Propafenone Before and After Phenobarbital Treatment

4.5.6.1 Effect of Heparin on Protein Binding of Propafenone

In our study, the use of heparin (in saline) to flush the indwelling cannula after blood sampling was necessary to prevent the formation of clots in the cannula. It is well known that the use of heparin affects the protein binding of a number of acidic drugs such as warfarin [Routledge *et*

a1., 1979] and digoxin [Storstein and Janssen, 1976]. Heparin *in vivo* releases lipoprotein lipases and increases the serum concentration of non-esterified fatty acid [Olivecrona et al., 1977]. The elevated free fatty acid compete with the drug for the protein binding site(s) on albumin, resulting in an overestimation of drug free fraction. Brown et al. [1981] have shown that the heparin-induced drug protein binding changes are, to a large extent, *in vitro* artifacts resulting from the continued *in vitro* activity of triglyceride lipases. The plasma protein binding of certain basic drugs, such as propranolol [Wood et al., 1979a], quinidine [Nilsen et al., 1977; Kessler et al., 1979] and verapamil [Keefe et al., 1981], have been shown to be affected by the use of heparin. Changes in concentrations of non-esterified fatty acid seem to affect the plasma protein binding of drugs in which albumin is the major binding protein, but not drugs which bind mainly to AAG [Grossman et al., 1982]. Although propranolol, quinidine and verapamil bind to AAG, they are probably primarily bound to serum albumin. The effect of heparin administration on the binding of PF has not been examined. However, the plasma protein binding of propranolol had been shown to be unaffected by heparin administration in doses necessary to maintain the function of indwelling catheters even when non-esterified fatty acid concentration was increased as a result of the lipolytic activity of heparin [Silber et al., 1980]. In our study, only a small amount of heparin (5 units) contained in sterile normal saline for injection was administered after each blood sample was collected and as a result, an effect on PF binding would not be anticipated. Alternatively, it is likely that the effect of heparin on the plasma protein binding of drugs is more important when very large doses of heparin (several thousand units in a few hours) are required in

cardiopulmonary bypass surgery or in hemodialysis [Naranjo *et al.*, 1980a, 1980b].

4.5.6.2 Effect of Phenobarbital Treatment on Propafenone Free Fraction

Studies on the effect of phenobarbital on the pharmacokinetics of propranolol in dog [Bai and Abramson, 1982; Vu *et al.*, 1983] and monkey [Branch *et al.*, 1974] illustrated that the major determinants of hepatic clearance, such as intrinsic clearance, liver blood flow and protein binding, can be affected by enzyme induction. For example, Branch *et al.* [1974] found a 45% increase in liver blood flow after phenobarbital treatment in the monkey. There is no evidence that a similar change occurs in man. In animal studies, phenobarbital was found to cause a dramatic increase in plasma AAG concentration and a decrease in serum free fraction of propranolol in dogs [Bai *et al.*, 1982] and an increase in serum protein binding of desmethylinipramine in rats [Brinkschulte and Breyer, 1982]. In man, the effect of phenobarbital on serum AAG concentration and drug protein binding is not clear. For example, Routledge *et al.* [1981] found that epileptics treated with anticonvulsants had serum AAG concentrations approximately twice that observed in untreated healthy volunteers and the free fraction of lidocaine was reduced in the treated subjects. Conflicting results were observed in epileptics treated with either carbamazepine or phenobarbital [Bruguerolle *et al.*, 1984]. Our results showed that phenobarbital treatment did not cause any change in the protein binding (free fraction) of PF in man (Figure 39). In addition, no change in the serum concentration of AAG, which we have proved to be an important binding protein for PF [Chan *et al.*, 1989], was shown after phenobarbital administration (Figure 40). This is in agreement with the finding of Kapil

et al. [1987] that phenobarbital (also three weeks treatment) caused no significant change in either serum AAG concentration or the free fraction of disopyramide in man. Similar results were also observed by Herman et al. [1983] on the binding of β -adrenoceptor blocker. Interspecies variations in the response of induction of protein synthesis [Branch and Herman, 1984] or seizure-induced changes in AAG concentration unrelated to drug treatment may explain the apparent discrepancy.

The volume of distribution indicates the extent of distribution and binding of the drug in the body. Since phenobarbital treatment did not affect the serum protein binding and free fraction of PF, the increase in V_{darea}/F or V_{dss}/F was likely due to the decrease in systemic availability (assuming that there was no change in the distribution and binding of PF in tissues). The decrease in systemic availability of PF after phenobarbital treatment was responsible for the marked reductions in C_{max} and AUC.

4.6 Phenobarbital Treatment in Healthy Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

4.6.1 Data Fitting

The values of pharmacokinetic parameters of PF were obtained after fitting the serum concentration *versus* time data of PF of the eight healthy smokers into AUTOAN.

4.6.2 Interindividual Variation in Kinetic Parameters of Propafenone

Like the non-smoking subjects, the smoking subjects also showed a large interindividual variation in the pharmacokinetic parameters of PF

(Table 20). Two of the smoking subjects were 'slow' metabolizers ($CL_{int} \leq 0.5$ L/min) and contributed to the considerable interindividual variation. Even when the data of these two subjects were excluded, the large interindividual variation still existed. Smoking is added as another important environmental factor which affects the disposition of drugs in the smoking population. However, smoking is not an 'independent' factor itself. It has been demonstrated that cigarette smoking is associated with age and alcohol consumption [Vestal and Wood, 1980; Sellers *et al.*, 1983]. In our study, smoking subjects were also carefully selected employing the same criteria as the non-smoking subjects. Their cigarette consumption was based on the number of cigarettes smoked per day (at least 20 cigarettes per day for the past 5 years). However, the extent of effect that smoking has on drug disposition is modified by factors that determine 'exposure' to cigarette smoke constituents. These include tobacco characteristics (e.g. harvesting and curing process), smoking characteristics (e.g. burn temperature), filter performance (e.g. material and design), smoking profile (e.g. puff frequency and duration), smoking style (e.g. pulmonary retention time) and constituent kinetics (e.g. bioavailability) [Sellers *et al.*, 1983].

Excluding the data of the two 'slow' metabolizers (subjects GE and MG), there was still a large interindividual variation in CL_{int} control of PF in the smoking subjects, ranging from 8.0 to 18.2 L/min (2.3 fold difference) (Table 20). Long term diet and other environmental factors may have also contributed to this large intersubject variation. Enzyme induction did not convert subject GE to a 'rapid' metabolizer (CL_{int} was 0.4 L/min before and 0.5 L/min after phenobarbital treatment). Although the CL_{int} of subject MG was increased to 1.0 L/min after phenobarbital

treatment, it was still low compared to the CL_{int} control of most 'rapid' metabolizers. In other words, after enzyme induction, subjects GE and MG still displayed long elimination half-life, high serum concentration, low oral clearance and absence of detectable 5-hydroxy PF, which are characteristics of a 'slow' metabolizer.

4.6.3 Induction of Phenobarbital Metabolism by Phenobarbital

Similar results were observed in the smokers as in the non-smokers. The decrease in systemic availability of PF as a result of enhanced first-pass metabolism by phenobarbital treatment caused a remarkable reduction in the serum levels, C_{max} and AUC of PF (Table 20). The increase in oral clearance was not as large as that of the non-smokers, ranging from 23 to 450%. A decrease in the serum concentration of 5-hydroxy PF and a decrease in the renal excretion of the conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF was also observed after enzyme induction in the smokers.

Excluding the data of the two 'slow' metabolizers reduced the interindividual variation in the kinetic parameters of PF among the smoking subjects but had no significant influence on the ranges of the percent increase in CL_{int} and the percent decrease in C_{max} and AUC.

4.6.4 Salivary Excretion of Propafenone

Like non-smokers, the saliva concentrations of PF in the smokers fluctuated over the 48 h (Appendix 6). A negative and significant correlation also existed between saliva/serum PF concentration ratio and saliva pH (control, $r = -0.541$; phenobarbital-treated, $r = -0.387$; $p < 0.05$), indicating that an increase in the pH of saliva will cause a decrease in PF salivary concentration. Propafenone concentration in saliva was

considerably higher than PF serum free concentration (Table 24).

Propafenone salivary concentration was approximately 36% (control) and 40% (phenobarbital-treated) of PF serum total concentration, with large inter- and intraindividual variations (Table 22).

The estimated percent free PF in serum was also significantly higher than the values obtained from equilibrium dialysis (Table 24), indicating that factors other than the pK_a of PF or the pH of saliva and serum, also contributed to the excretion of PF in saliva. Unlike the non-smokers, the correlation between salivary concentration and serum free concentration is both close ($r = 0.920$) and significant. However, like the non-smokers, there was also a better correlation between serum total concentration and serum free concentration ($r = 0.993$, $p < 0.001$) than between serum total concentration and salivary concentration ($r = 0.940$, $p < 0.001$) or between salivary concentration and serum free concentration ($r = 0.940$, $p < 0.001$) (Figure 45).

4.7 Extent of Metabolic Induction of Propafenone by Phenobarbital in Healthy Non-smokers and Smokers

The dose and the duration of treatment of the enzyme inducing agent are essential in eliciting maximal enzyme induction [Breckenridge *et al.*, 1972; Mignet *et al.*, 1977; Ohnhaus *et al.*, 1977; Ohnhaus and Park, 1979; Ohnhaus *et al.*, 1983]. When the dose is too low or when the duration of treatment is too short, significant induction may not be observed [Breckenridge *et al.*, 1972; Eichelbaum *et al.*, 1986]. In general, the extent of enzyme induction depends on the elimination rate of the enzyme

inducing agent and the enzyme turnover rate. Microsomal enzyme turnover rate (ranged from 1-6 days [Lai et al., 1978]), as well as the dose and duration of treatment, are more important on the reversal of enzyme induction. Enzyme induction persists as long as the enzyme inducing agent is administered. The elimination half-life of phenobarbital is about 24 h. Since it takes about one week (five to seven times the half-life) for phenobarbital serum concentration to reach steady-state, a minimum of two weeks is necessary for maximal induction of microsomal enzymes. In order to ensure maximal induction, we treated our subjects with phenobarbital at a therapeutic dose of 100 mg daily for three weeks. Blood samples were obtained periodically to ensure serum phenobarbital concentration had reached steady-state and was between 10-20 $\mu\text{g/mL}$ at the second stage of the study for all subjects.

Several authors [Vesell and Page, 1969; Breckenridge et al., 1971; Breckenridge and Orme, 1971] have drawn a general conclusion that subjects who metabolize drugs slowly will show a greater enzyme induction than subjects who metabolize drugs rapidly. Our findings do not support the conclusion drawn by these authors. The decrease in AUC after phenobarbital treatment ranged from 9 to 89% in the non-smoking subjects (Table 13) and 19 to 82% in the smoking subjects (Table 20). The two non-smokers who had the lowest (9%, subject DA) and the highest (82%, subject CA) metabolic induction had similar $\text{CL}_{\text{int control}}$ (8.3 and 7.5 L/min, respectively), a parameter that indicates the individual's ability to metabolize drug. The smoking subject who had the highest metabolic induction (82%, subject DW) was not a 'slow' metabolizer. On the other hand, the two 'slow' metabolizers (subject GE and MG) did not show a greater enzyme induction (22 and 46% respectively) compared to the 'rapid' metabolizers.

Furthermore, $CL_{int\ control}$ did not correlate with the percent decrease in AUC in both non-smoking ($r = -0.425$, $p > 0.05$) and smoking subjects ($r = 0.319$, $p > 0.05$) (combined non-smokers and smokers, $r = 0.048$, $p > 0.05$) (Figure 47).

Two hypotheses concerning the intersubject variation in the extent of metabolic induction between subjects have been proposed. One was that following induction there will be a smaller intersubject variation in drug metabolising activity than prior to induction. This hypothesis is an interpretation of the conclusion drawn by the previous mentioned authors [Vesell and Page, 1969; Breckenridge *et al.*, 1971; Breckenridge and Orme, 1971]. The other hypothesis was that the range of intersubject variation in drug metabolising activity should be as large after induction as before induction [Herman *et al.*, 1982]. Our study supports the latter hypothesis. In fact, the coefficient of variation in CL_{int} in the non-smokers after induction (119%) was twice that before induction (61%) (Table 13) while the coefficient of variation in CL_{int} in the smokers before and after induction was similar, including (76% before and 82% after) or excluding (42% before and 50% after) the two 'slow' metabolizers (Table 20).

Another implication of the conclusion drawn by the previous mentioned authors [Vesell and Page, 1969; Breckenridge *et al.*, 1971; Breckenridge and Orme, 1971] is that subjects who metabolize drugs slowly will show a higher serum phenobarbital concentration and a greater percent reduction in AUC, provided PF and phenobarbital are metabolized by the same or similar route or their metabolism is under a common regulatory control (both undergo metabolism *via* aromatic hydroxylation). Our data demonstrated that although phenobarbital treatment caused a reduction in C_{max} and AUC of PF, there was no apparent correlation between serum phenobarbital concentration

and induction of PF metabolism, as expressed by $[(AUC_P - AUC_C)/AUC_C] \times 100\%$ ($r = -0.323$, $p > 0.05$) (Figure 46). Different subjects (NP and CA) required different serum concentrations of inducing agents to produce the same induction effect (64%). Although some subjects (e.g. subjects GE and MA) produced similar induction effects (20%) with similar serum phenobarbital concentration (20 $\mu\text{g/mL}$), others (e.g. subjects CA and SG) produced a different induction effect (64 and 9%) with identical serum phenobarbital concentration (13 $\mu\text{g/mL}$). Similar results were found between the extent of induction of alprenolol metabolism by specific pentobarbital plasma concentrations [Collste *et al.*, 1979] and likewise the extent of induction of warfarin metabolism by quinalbarbitone plasma concentrations [Breckenridge *et al.*, 1972]. The poor correlation between the extent of induction of PF by phenobarbital suggests that PF and phenobarbital are metabolized by different isozymes of cytochrome P-450.

Kellermann and Luyten-Kellermann [1977] studied the effect of enzyme induction by phenobarbital on antipyrine half-lives. These authors suggested that interindividual variation in the metabolic rate of the inducing agent should be taken into account when conducting induction studies in order to obtain comparable results in all subjects. However, in their studies, even after the dose of phenobarbital was adjusted to the individual rates of metabolism of the inducing agent, they could not find any correlation between the initial antipyrine half-life and the percent decrease in plasma half-life. These authors also suggested that other factors such as interindividual differences in the protein binding or differences in renal excretion of phenobarbital may contribute to the observed variation in enzyme induction. They concluded that since the decrease in antipyrine half-lives ranged from 13.3 to 30.6%, the

interindividual variation in the magnitudes of phenobarbital-evoked induction may be under genetic control. Considerable interindividual variability was also observed in the induction of metoprolol metabolism by pentobarbital (2-46% decrease in AUC) [Haglund *et al.*, 1979] and in the induction of warfarin metabolism by quinalbarbital (5-64.5% decrease in steady-state plasma warfarin concentration) [Breckenridge *et al.*, 1972]. Both non-smoking and smoking subjects in our study showed a similar wide range of percent decrease in C_{max} (0-87% in the non-smokers and 8-85% in the smokers) and AUC (9-89% in the non-smokers and 19-82% in the smokers) after phenobarbital treatment, indicating that phenobarbital induces PF metabolism to a similar extent in both non-smoking and smoking populations. Since we have shown that the decrease in AUC did not correlate with the individual's CL_{int} or serum phenobarbital concentration, it seems possible that such variations were under both environmental and genetic control.

4.8 Effect of Cigarette Smoking on the Pharmacokinetics and Serum Protein Binding of Propafenone

4.8.1 Statistical Analysis

In the analysis of the difference in the kinetic parameters between the non-smoking and the smoking subjects, the Mann-Whitney test with tied ranks was used. It is a nonparametric analogue of or alternative to the unpaired t-test. Initial F test showed that there were significant differences in the variances in C_{max} and AUC between the non-smokers and the smokers. The unpaired t-test was, therefore, not applicable since the unpaired t-test assumes equality of the variances of the two sampled

populations [Zar, 1984].

4.8.2 Effect of Cigarette Smoking on the Metabolism of Propafenone

Table 26 allows a comparison of the kinetic parameters of PF between eight non-smoking subjects and eight smoking subjects or six smoking subjects excluding the two 'slow' metabolizers. Comparing the values of the kinetic parameters in the control state, the mean C_{\max} in the smokers (107 ng/mL) was about half of that of the non-smokers (253 ng/mL) while the AUC in the smokers (405 h.ng/mL) was three fold smaller than that of the non-smokers (1360 h.ng/mL). In addition, the mean CL_{int} in the smokers (13.2 L/min) was double that of the non-smokers (6.1 L/min). The mean salivary AUC in the smokers (117.9 h.ng/mL) was also smaller than that of the non-smokers (190.2 h.ng/mL). While smoking did appear to increase the clearance of PF, it is difficult to conclude that smoking induced the metabolism of PF, due to the small sample size and the lack of comparison of smokers (serving as their own experimental control) under a nonsmoking circumstance.

Table 27 provides a comparison of the kinetic parameters of 5-hydroxy PF between the non-smoking and the smoking subjects. Since there was no significant difference in both C_{\max} and AUC of 5-hydroxy PF between the non-smokers and smokers, aromatic hydroxylation was probably not induced by cigarette smoking.

4.8.3 Effect of Cigarette Smoking on the Serum Protein Binding of Propafenone

In addition to drug-metabolizing capacity, smoking may also affect drug protein binding. The extent of lidocaine binding was found to be

greater in serum obtained from smokers than in the serum of non-smokers [McNamara *et al.*, 1980]. This was probably due to the elevated serum AAG concentration in the smoking population [Hollinshead *et al.*, 1977; Benedek *et al.*, 1983]. Benedek *et al.* [1983] reported that the mean serum AAG concentration in smokers (mean = 84.3 mg/dL, n = 10) was significantly different from that of non-smokers (mean = 62.8 mg/dL, n = 10). However, they also found that in contrast to the strong correlation between AAG serum level and protein binding of propranolol in non-smokers, the propranolol unbound fraction did not reflect elevated serum AAG concentration. They suggested that other factors, including different serum concentration of lipoprotein and albumin, may affect the binding of propranolol in smokers. Alternatively, components in cigarette smoke may compete with propranolol for the serum protein binding sites. The underlying mechanism(s) behind the elevation in serum AAG concentration in the smoking population is unknown. Benedek *et al.* [1983] suggested that smoking may cause an increase in this acute phase reactant protein either in a fashion similar to that of disease states or by an enzyme induction mechanism. On the other hand, the finding that smoking did not affect the protein binding of phenytoin [Benedek *et al.*, 1983; Rose *et al.*, 1978] was not surprising since phenytoin is an acidic drug which binds to albumin so elevated serum AAG concentrations should not affect its binding. Our results did not support the presence of elevated AAG concentration in the serum of healthy heavy cigarette smokers. There were no significant differences in either serum AAG concentration or PF free fraction between the non-smoking and the smoking subjects.

4.9 Concentration-Response Relationship of Propafenone

4.9.1 Signal Averaged Electrocardiograms

The problem associated with normal surface ECG is a high signal to noise ratio, resulting in variability in the measurement of QRS width from beat to beat. In the present study, a ECG signal averaging technique was used. It is a tracing which results from the computerized averaging of a large number of heart beats. The signal to noise ratio decreases in proportion to the square root of the number of beats averaged. In general, it is possible to accurately measure QRS width within one millisecond by averaging 80 or more beats. In our study, we averaged 150 beats to generate the ECG data and to obtain an accurate measurement of QRS width.

4.9.2 Determinants of the Pharmacological Effect of Propafenone

The correlation between PF serum total concentration and QRS width was not significant. It seems likely that for PF, free drug concentration, rather than total drug concentration, would better correlate with pharmacological effect. It could also be that more than one factor may contribute to or influence the pharmacological response of PF and account for the observed poor concentration-response relation.

Already discussed in Section 4.4.4. is the observation that PF appears to be a drug which may benefit from the assessment of free drug concentrations in serum. In order to justify free drug level monitoring, the correlation between therapeutic or toxic response and free drug concentration has to be established [Barre *et al.*, 1988]. The free drug concentrations of two samples from each patient were determined and found not to significantly correlate with QRS width. However, the correlation

between serum PF free concentration and QRS width ($r = 0.346$) was better than the correlation between serum PF total concentration and QRS width ($r = 0.288$). The serum AAG concentration of the ten patients were within the normal range. Except for the two 'slow' metabolizers who had a steady-state serum PF concentration of $1.8 \mu\text{g/mL}$, the steady-state serum concentrations of the other patients were below $1.5 \mu\text{g/mL}$ (range 0.17 - $0.92 \mu\text{g/mL}$). Since PF displayed concentration-independent binding below $1.5 \mu\text{g/mL}$, it was possible that for these reasons the correlation between PF serum free concentration and QRS width in these patients was not apparent.

The major metabolite of PF, 5-hydroxy PF, has been found to be pharmacologically active in animal studies [Philipsborn *et al.*, 1984; Valenzuela *et al.*, 1987; Thompson *et al.*, 1988]. Although its precise pharmacological effect is unclear in man, this metabolite has been found to accumulate in the plasma of patients who received propafenone for the treatment of arrhythmia [Kates *et al.*, 1985]. The steady-state serum concentration and AUC of 5-hydroxy PF has been shown to be 1.5-6 times lower than those of PF (Table 28). However, if 5-hydroxy PF is also pharmacologically active in man, it can still contribute to the overall antiarrhythmic effect of PF and to the apparently poor correlation observed between serum PF concentration and its antiarrhythmic effect.

We have shown that the binding ratio of PF is significantly correlated to serum AAG concentration in sera from patients with chronic renal failure. We have further demonstrated a significant correlation between the free fraction of PF and serum AAG concentration in patients with arrhythmias (Figure 51). Even in healthy subjects, there is a wide interindividual variation in the serum concentration of AAG, ranging from

33 to 137 mg/dL [Routledge *et al.*, 1980]. This variation is further magnified by certain physiological and pathological states [Piafsky *et al.*, 1978] and concomitant drug therapy [Baumann *et al.*, 1982], which may increase or decrease serum AAG concentration. Diurnal variation, on the other hand, accounts for some intraindividual variation in serum AAG concentration [Yost and DeVane, 1985]. The relationship between PF free fraction and AAG appears to be curvilinear (Figure 51), suggesting that a small change in AAG concentration may bring about a large alteration in PF free fraction. Therefore, in addition to PF serum total concentration and 5-hydroxy PF serum concentration, serum AAG concentration which affects PF serum free concentration may also be an important factor that influences the pharmacological effect of PF.

Using multiple stepwise regression, we have demonstrated that it is possible to develop an equation to predict the QRS width as a function of all the factors that may affect or contribute to the overall pharmacological response of PF, including serum PF concentration, serum 5-hydroxy PF concentration and serum AAG concentration. The QRS width predicted from using Equation 8 is, in general, lower than but significantly correlated with the measured QRS width ($r = 0.536$). Although it is more common to use the logarithm of serum drug or metabolite concentration in the evaluation of the concentration-response relationship, the QRS width predicted from Equation 9 is closer to and significantly correlated with the measured QRS width ($r = 0.630$).

4.9.3 Polymorphic Oxidative Metabolism

Patients who are 'slow' metabolizers of PF appear to exhibit a different concentration response relationship from that observed in 'rapid'

metabolizers [Siddoway *et al.*, 1987]. While the reason(s) for this are not clearly established, it is interesting to note that the 'slow' metabolizers exhibit apparently higher PF serum concentrations, a smaller oral clearance and an absence of serum 5-hydroxy PF. Furthermore, unlike the disproportional dose-concentration relationship observed in 'rapid' metabolizers, 'slow' metabolizers also demonstrate what appears to be a proportional dose-concentration relationship [Siddoway *et al.*, 1987]. The developed equations to calculate QRS width apparently only apply to 'rapid' metabolizers. Therefore, it is necessary to identify a patient's phenotype before using these equations. The 'slow' metabolizers can be identified simply by measuring their dose-corrected steady-state plasma concentration of PF (>2 ng/mL/mg daily PF dose) or by checking for the absence of plasma 5-hydroxy PF [Siddoway *et al.*, 1987].

5. SUMMARY AND CONCLUSION

5.1 Capillary Electron-Capture Detection Gas-Liquid Chromatographic Analysis of Propafenone

The GLC-ECD method developed for the quantitation of PF was the first GLC method employing fused-silica capillary column and splitless injection technique. Propafenone was derivatized with HFBA to increase selectivity and sensitivity and the functional groups involved in the derivatization reaction, including hydroxyl and secondary amine, were confirmed by GLC-MS. Linearity was observed in the ranges 0.5-10 ng/mL (saliva samples, single-dose study), 2.5-50 ng/mL (serum samples, single-dose study) and 10-100 ng/mL (serum samples, steady-state study). The coefficient of variation was found to be less than 10% over the concentration ranges studied. The accuracy of the GLC-ECD method was confirmed by comparing PF serum concentrations of patient samples with the developed method and a published HPLC method. The limit of determination was 2.5 ng/mL using 1 mL of serum. The sensitivity and selectivity of the GLC-ECD method allowed us to accurately quantitate PF in serum, saliva and dialysate in single-dose pharmacokinetic studies and protein binding studies of PF. The reproducibility and repeatability of the method made it possible to carry out routine analysis of PF during plasma total (or free) drug monitoring of patients receiving PF for treatment of arrhythmias.

The GLC-ECD method developed for the quantitation of PF was further modified to measure the major and active metabolite of PF, 5-hydroxy PF. Linearity was observed in the range 2.5-50 ng/mL. The coefficient of variation was found to be less than 3.5%.

5.2 *In Vitro* Serum Protein Binding Study

The results of the *in vitro* protein binding study of PF can be summarized as follows:

i) Using serum obtained from healthy human subjects, we confirmed *in vitro* the concentration-dependency of the serum protein binding of PF. The free fraction of PF was 0.027 ± 0.011 at a PF concentration of $0.25 \mu\text{g/mL}$, 0.041 ± 0.010 within the therapeutic concentration range ($0.5\text{-}2 \mu\text{g/mL}$), 0.138 ± 0.012 at a PF concentration of $25 \mu\text{g/mL}$ and 0.187 ± 0.005 when the PF concentration was increased to $100 \mu\text{g/mL}$. No evidence for significant concentration-dependent changes in free fraction was observed within the PF concentration range of $0.25\text{-}1.5 \mu\text{g/mL}$. However, concentration-dependent binding was demonstrated at concentrations greater than $1.5 \mu\text{g/mL}$. A high-affinity, low-capacity binding site ($K_1 = 6.53 \times 10^5 \text{ M}^{-1}$; $n_1P_1 = 1.73 \times 10^{-4} \text{ M}$) and a low-affinity, high-capacity binding site ($K_2 = 8.77 \times 10^3 \text{ M}^{-1}$; $n_2P_2 = 8.57 \times 10^{-3} \text{ M}$) were identified.

ii) In pooled uremic serum, the free fraction of PF was approximately 50% of that of normal serum throughout the concentration range studied ($1\text{-}5 \mu\text{g/mL}$).

iii) In serum obtained from patients with chronic renal failure, the increase in PF binding ratio was associated with the increase in serum AAG concentrations. The positive and significant ($p < 0.05$) correlation between these two parameters ($r = 0.8302$) suggested that AAG was an important binding protein for PF in serum.

5.3 Phenobarbital Treatment in Healthy Non-smokers and Smokers: Pharmacokinetics and Binding Studies of Propafenone and 5-Hydroxy Propafenone

The effect of enzyme induction on the pharmacokinetics of PF and its major and active metabolite, 5-hydroxy PF can be summarized and concluded as follows:

i) There was considerable interindividual variability in the pharmacokinetic parameters, including C_{\max} , CL_{int} and AUC of serum PF, saliva/serum concentration ratio and AUC of salivary PF and C_{\max} and AUC of 5-hydroxy PF before and after phenobarbital treatment. Because of the considerable interindividual variation in these parameters, each subject served as his own control.

ii) Except for the two smokers who were 'slow' metabolizers, all non-smoking and smoking subjects were 'rapid' metabolizers ($CL_{\text{int}} > 0.5$ L/min). The two 'slow' metabolizers were characterized by low intrinsic clearance, high PF serum concentration, low oral clearance and undetectable 5-hydroxy PF in serum.

iii) Phenobarbital treatment (100 mg daily for 23 days) induced hepatic microsomal enzymes and enhanced the extent of the first-pass metabolism of PF. There was a significant increase in CL_{int} after phenobarbital treatment. The increase in CL_{int} ranged from 10-831% (mean 194%) in the non-smokers and 23-450% (mean 156%) in the smokers. This resulted in a substantial decrease in the systemic availability, as shown by a significant reduction in C_{\max} and AUC. The decrease in C_{\max} ranged from 0-87% (mean 45%) in the non-smokers and 8-85% (mean 46%) in the smokers while the decrease in AUC ranged from 10-89% (mean 50%) in the non-

smokers and 19-82% (mean 49%) in the smokers.

iv) The percent decrease in C_{\max} was similar to the percent decrease in serum AUC for PF in the non-smokers and the smokers.

v) Except for two smokers, enzyme induction also caused a substantial reduction in the salivary AUC_0^t of PF. The decrease in AUC_0^t ranged from 1-82% (mean 44%) in the non-smokers and 30-81% (mean 54%) in the smokers.

vi) The percent decrease in salivary AUC_0^t of PF mirrored the percent decrease in serum AUC in the non-smoking and the smoking subjects.

vii) Enzyme induction did not affect $t_{1/2\beta}$ or t_{\max} of PF in the non-smokers or the smokers.

viii) Twenty-three days of phenobarbital treatment did not cause any change in PF free fraction or serum AAG concentration in the non-smokers and the smokers.

vx) There was a wide range in the extent of metabolic induction of PF by phenobarbital, as expressed by the percent decrease in AUC, in the non-smokers and the smokers, and in 'rapid' and 'slow' metabolizers.

x) Enzyme induction did not convert 'slow' metabolizers of PF to 'rapid' metabolizers.

xi) The extent of metabolic induction of PF by phenobarbital was independent of the serum concentration of the inducing agent, phenobarbital, or of the initial ability of the individual's liver to metabolize drugs (CL_{int} control).

xii) The poor correlation between the extent of metabolic induction of PF by phenobarbital suggested that PF and phenobarbital were metabolized by different isozymes of cytochrome P-450.

xiii) Phenobarbital treatment decreased the serum concentration,

C_{\max} and AUC of 5-hydroxy PF. The decrease in C_{\max} ranged from 17-60% (mean 40%) in the non-smokers and 19-71% (mean 52%) in the smokers while the decrease in AUC ranged from 24-81% (mean 45%) in the non-smokers and 48-72% (mean 60%) in the smokers.

xiv) Phenobarbital treatment also decreased the renal excretion (expressed as percent of dose) of the conjugates of 5-hydroxy PF and 5-hydroxy-4-methoxy PF.

xv) The metabolic pathway of PF which was expected to be induced by phenobarbital could not be identified through our findings. Several possibilities have been discussed. The aromatic hydroxylation pathway may be noninducible while glucuronidation may or may not be induced. Biliary excretion of PF may be increased by phenobarbital treatment. In such case, an induction in glucuronidation may not be observed since an increase in the glucuronide conjugates of 5-hydroxy PF would appear in the bile or the feces and not in the urine. The decrease in 5-hydroxy PF after enzyme induction could also be the result of induction of other minor metabolic pathways of PF.

xvi) Compared to the non-smokers, heavy cigarette smokers had a significantly larger CL_{int} , a lower C_{\max} and a smaller AUC (Mann-Whitney test with tied ranks, $p > 0.05$).

xvii) While smoking did appear to increase the clearance of PF, it is difficult to conclude that smoking induced the metabolism of PF, due to the small sample size and the lack of comparison of smokers (serving as their own experimental control) under a nonsmoking circumstance.

5.4 Evaluation of Propafenone Serum Concentration-Response Relationship

The study of the serum concentration-response relationship of PF can be summarized and concluded as follows:

i) The QRS width measured from the signal averaged ECG data is an accurate and easily obtained measure of the PF antiarrhythmic effect.

ii) Several factors such as PF serum concentration, 5-hydroxy PF serum concentration and serum AAG concentration may contribute to or influence the overall pharmacological effect of PF. We have demonstrated that it was possible to develop an equation to include all these factors to predict the QRS width. The equation, developed using multiple stepwise regression, was described as $Y = 0.5X_1 + 4.5X_2 + 347X_3 + 79$ where Y is QRS width, X_1 is log PF serum concentration, X_2 is log 5-hydroxy PF serum concentration and X_3 is the reciprocal of serum AAG concentration.

iii) Unlike the usual concentration-response relationship which uses the logarithm of serum drug concentration, serum concentration of PF and 5-hydroxy PF, rather than the logarithm of these terms, yielded a better estimate of QRS width. The equation was described as $Y = 0.004X_1' + 0.04X_2' + 379X_3 + 95$ where Y is QRS width, X_1' is PF serum concentration, X_2' is 5-hydroxy PF serum concentration and X_3 is the reciprocal of serum AAG concentration.

In conclusion, clinical monitoring of PF may be necessary in patients receiving this drug for treatment of arrhythmias since PF shows remarkable interindividual variation. It may also be necessary to identify the patient's phenotype since 'slow' metabolizers have different pharmacokinetic characteristics of PF and an undetectable level of 5-hydroxy PF. 'Slow' metabolizers can be simply identified by their high

plasma PF concentrations or by the absence of 5-hydroxy PF in plasma. Patient monitoring may also be necessary in situations when the physiological and pathological state of the patient causes a dramatic increase in serum AAG concentration, which may result in an alteration in PF free fraction. Monitoring should also be considered in patients when PF is administered with other highly protein bound basic drugs which may displace PF from its protein binding sites or with other enzyme inducing agents which may enhance the first-pass metabolism of PF.

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7. APPENDIX

Appendix 1. Calculation of percent of free (unbound) drug in serum for a basic drug.

The Henderson-Hasselbalch equation for a basic drug is as follows:

$$\text{pH} = \text{pK}_a + \log (C_u/C_i)$$

$$\log (C_u/C_i) = \text{pH} - \text{pK}_a$$

$$C_i/C_u = 10^{\text{pK}_a - \text{pH}}$$

where K_a = dissociation constant

C_u = concentration of the unionic moiety

C_i = concentration of the ionic moiety

Concentration of free drug in saliva, C_f saliva

$$= C_u \text{ saliva} + C_i \text{ saliva}$$

$$= C_u \text{ saliva} + (C_u \text{ saliva} \times 10^{\text{pK}_a - \text{pH}_s})$$

$$= C_u \text{ saliva} (1 + 10^{\text{pK}_a - \text{pH}_s})$$

Concentration of free drug in serum, C_f serum

$$= C_u \text{ serum} + C_i \text{ serum}$$

$$= C_u \text{ serum} + (C_u \text{ serum} \times 10^{\text{pK}_a - 7.4})$$

$$= C_u \text{ serum} (1 + 10^{\text{pK}_a - 7.4})$$

where pH_s = pH of saliva

7.4 = pH of serum

$$R = \frac{C_f \text{ saliva}}{C_f \text{ serum}} = \frac{C_u \text{ saliva} (1 + 10^{\text{pK}_a - \text{pH}_s})}{C_u \text{ serum} (1 + 10^{\text{pK}_a - 7.4})}$$

Since $C_u \text{ saliva} = C_u \text{ serum}$ [Koysooko *et al.*, 1974; Huffman, 1975]

$$R = \frac{C_f \text{ saliva}}{C_f \text{ serum}} = \frac{(1 + 10^{\text{pK}_a - \text{pH}_s})}{(1 + 10^{\text{pK}_a - 7.4})}$$

Appendix 1. (Cont'd)

$$\frac{C_{f \text{ saliva}}}{C_{f \text{ serum}}} = \frac{C_{p \text{ saliva}} \times f_{\text{saliva}}}{C_{p \text{ serum}} \times f_{\text{serum}}}$$

where f_{saliva} = fraction of drug unbound in saliva
 f_{serum} = fraction of drug unbound in serum

$$\frac{C_{p \text{ saliva}} \times f_{\text{saliva}}}{C_{p \text{ serum}} \times f_{\text{serum}}} = \frac{1 + 10^{\text{pKa-pHs}}}{1 + 10^{\text{pKa-7.4}}}$$

Assume drug exists totally in the unbound form in saliva, $f_{\text{saliva}} = 1$

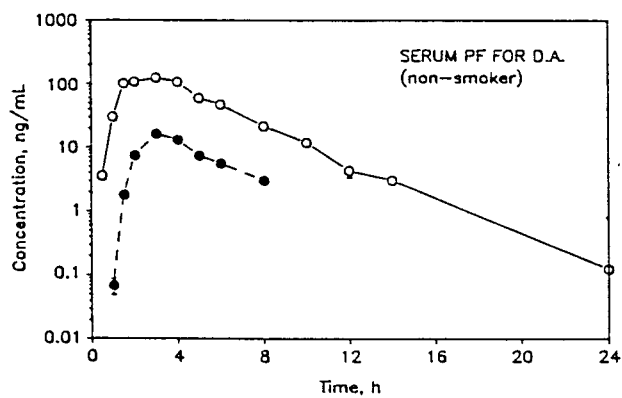
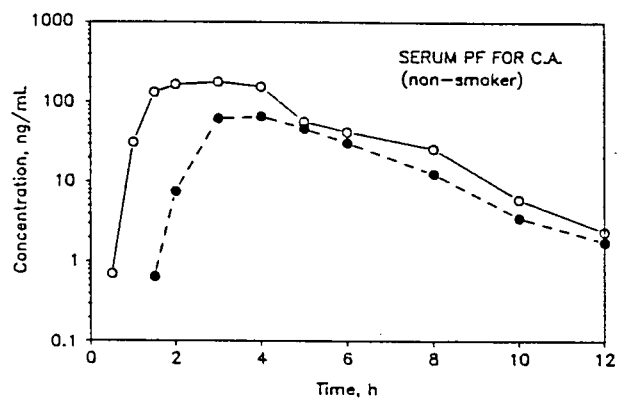
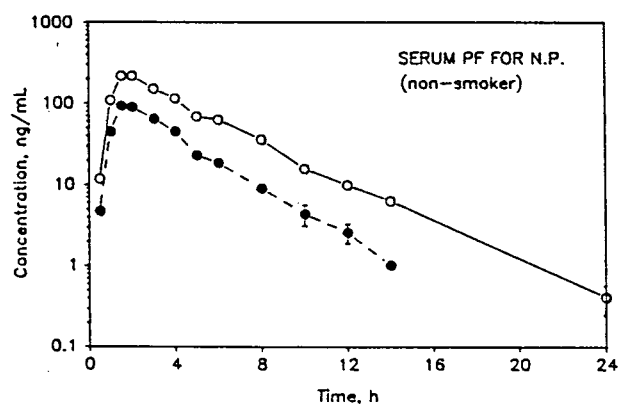
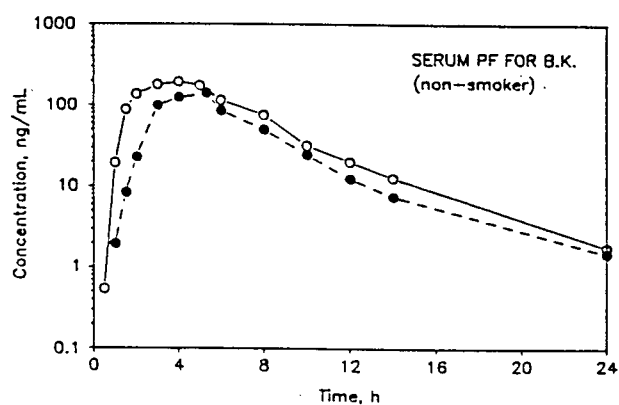
$$f_{\text{serum}} = \frac{C_{p \text{ saliva}}}{C_{p \text{ serum}}} \times \frac{1 + 10^{\text{pKa-7.4}}}{1 + 10^{\text{pKa-pHs}}}$$

Percent of free drug in serum

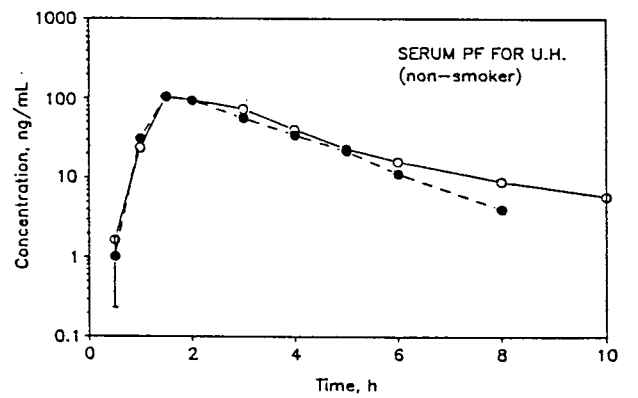
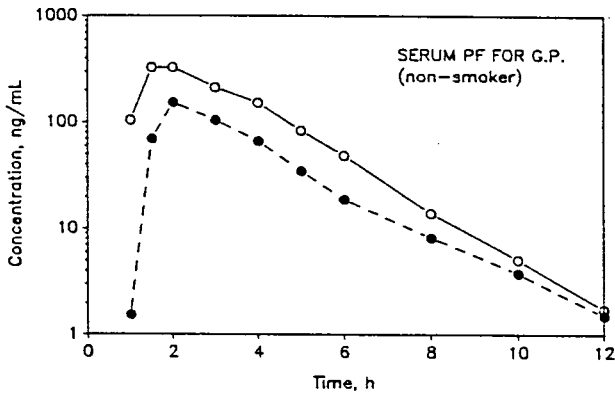
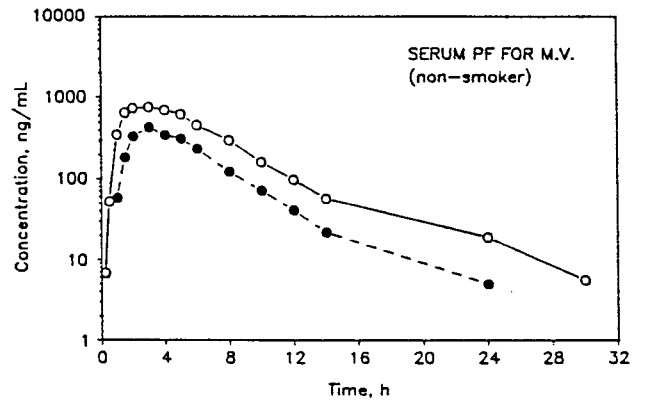
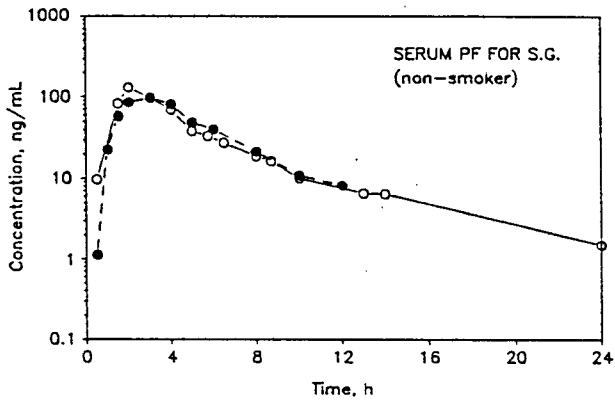
$$= f_{\text{serum}} \times 100$$

$$= \frac{C_{p \text{ saliva}}}{C_{p \text{ serum}}} \times \frac{1 + 10^{\text{pKa-7.4}}}{1 + 10^{\text{pKa-pHs}}} \times 100$$

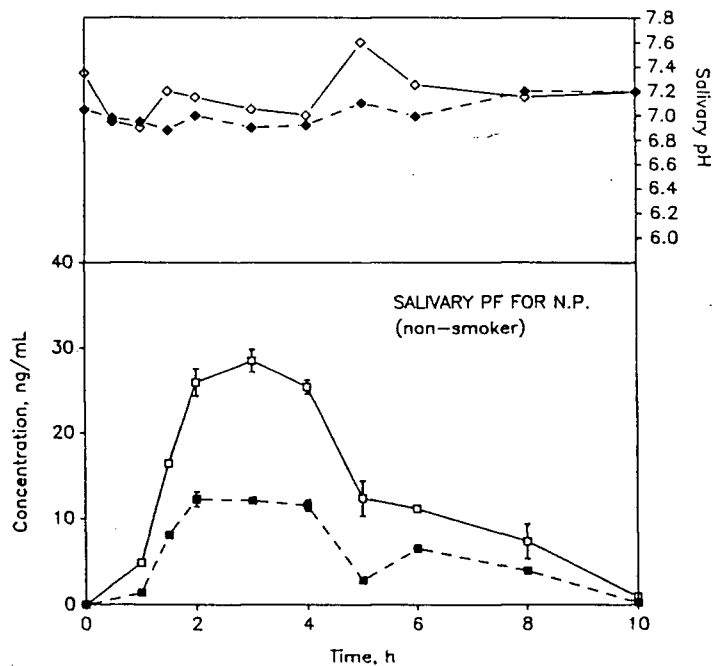
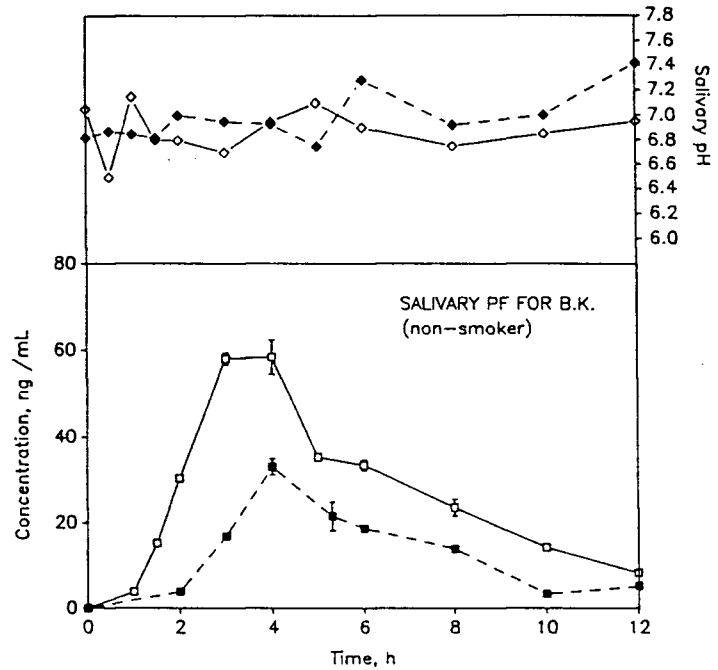
Appendix 2. Semi-logarithmic plots of the propafenone serum concentration *versus* time curves of eight non-smoking subjects before (O) and after 23 days of phenobarbital treatment (●).



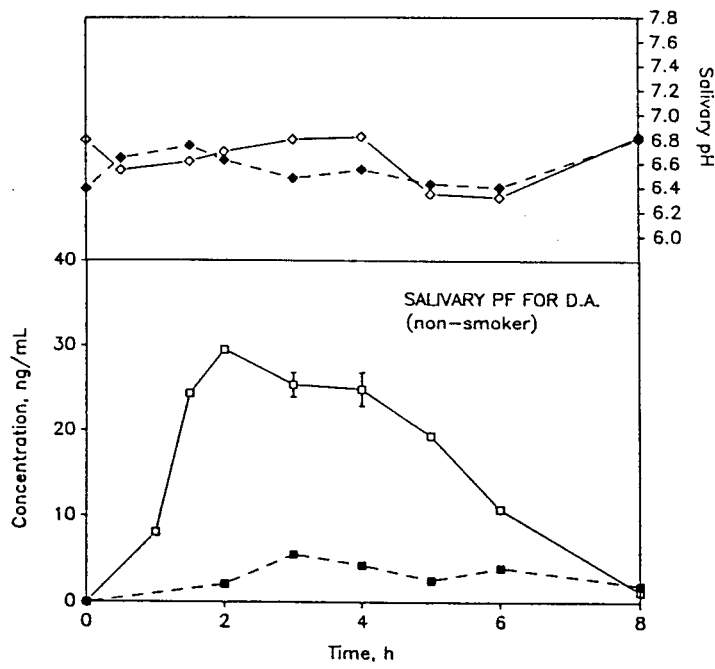
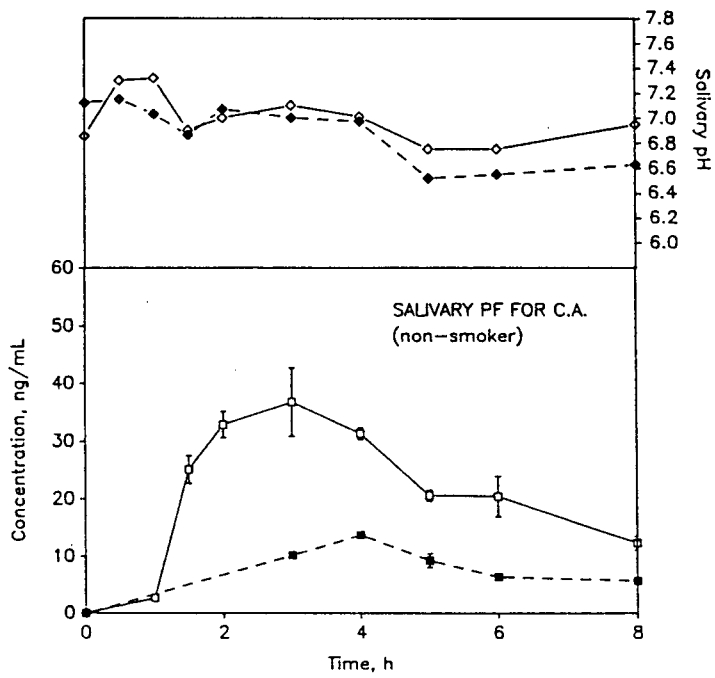
Appendix 2. (Cont'd)



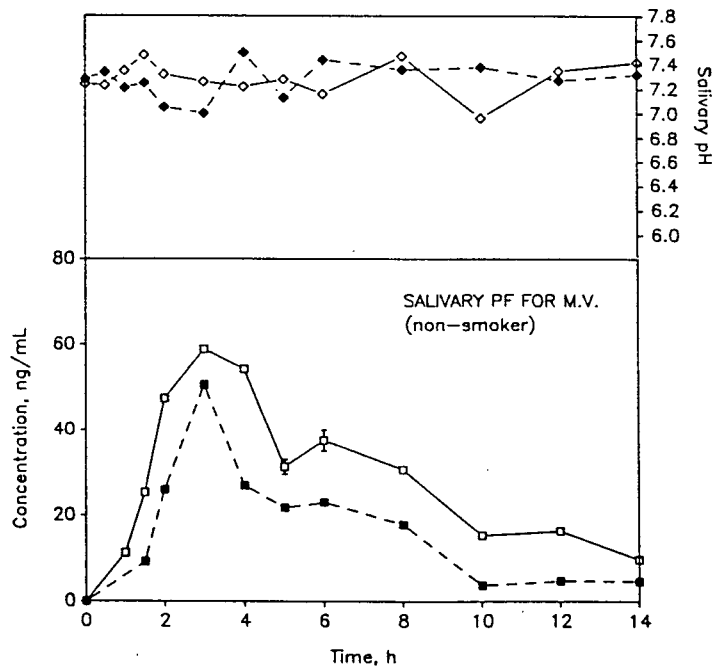
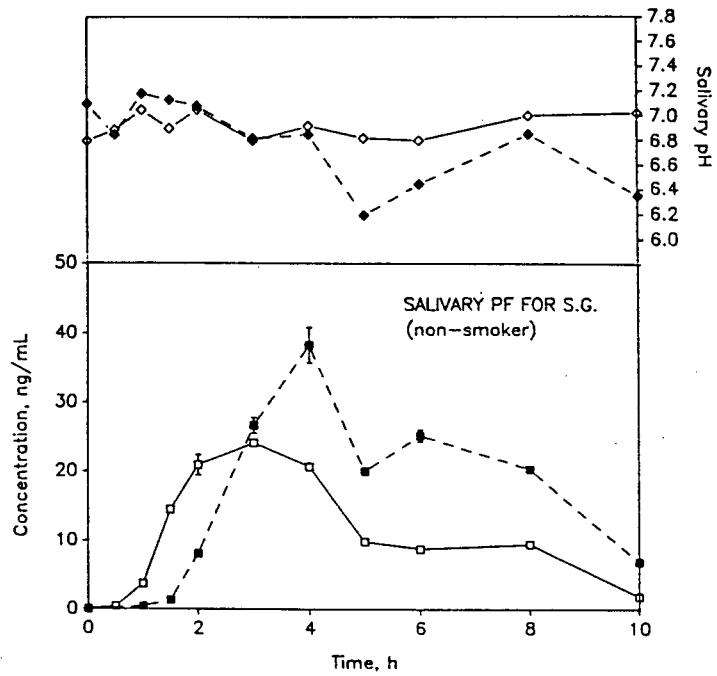
Appendix 3. Semi-logarithmic plots of the propafenone salivary concentration versus time curves of eight non-smoking subjects before (\square) and after 23 days of phenobarbital treatment (\blacksquare). Also shown in the figures is the pH of saliva before (\diamond) and after (\blacklozenge) phenobarbital treatment.



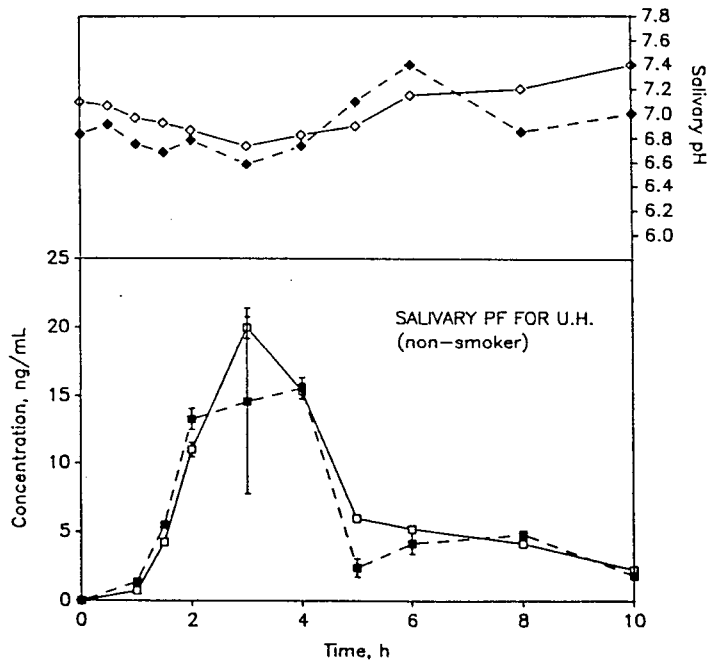
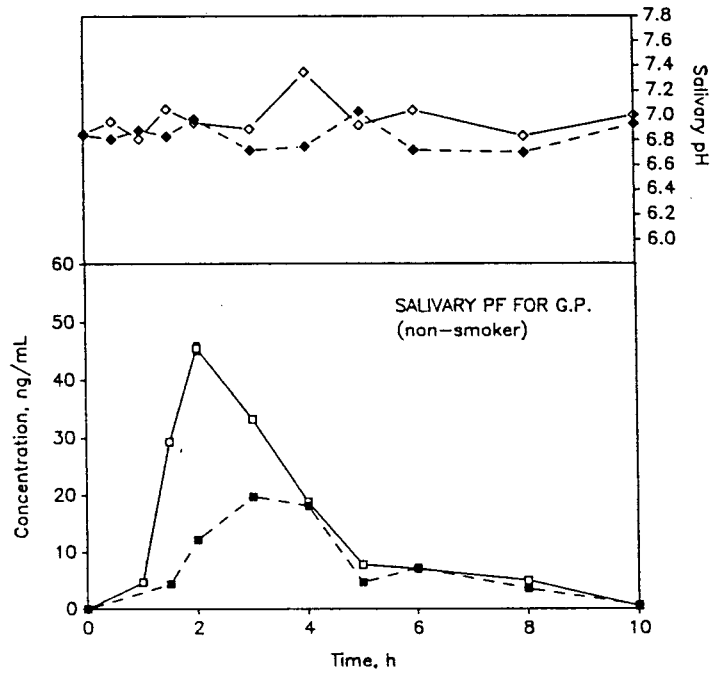
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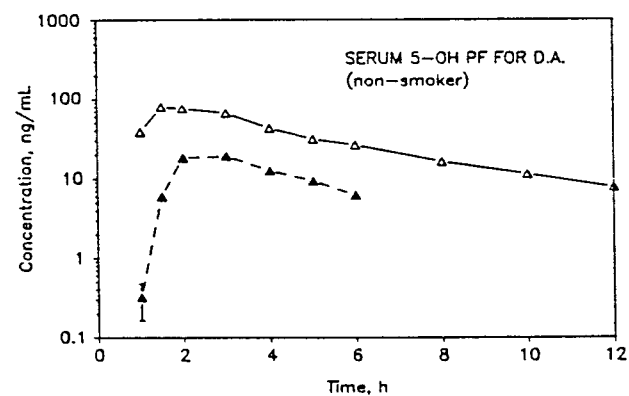
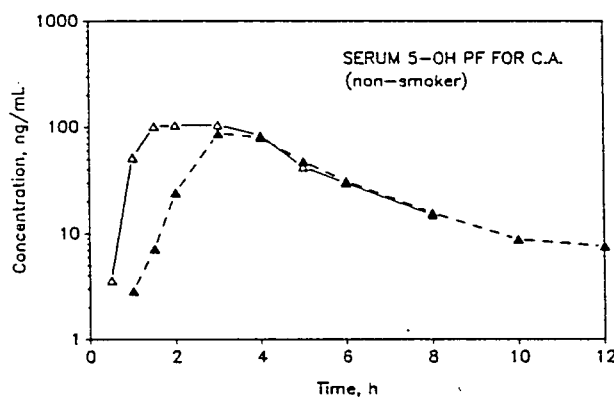
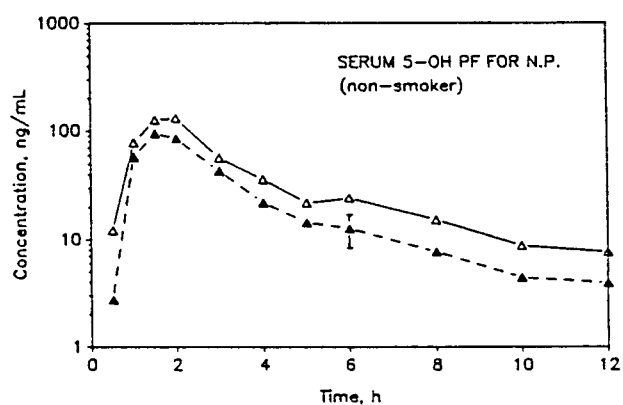
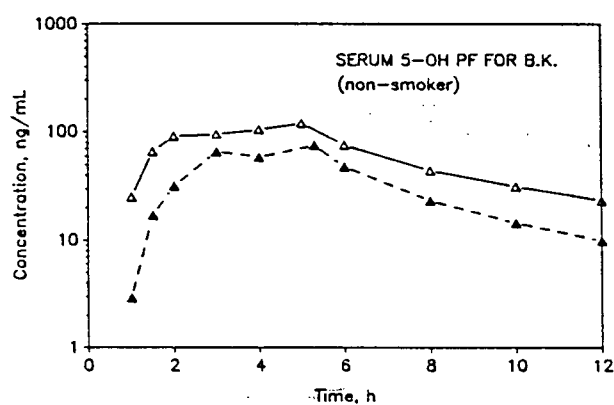
Appendix 3. (Cont'd)



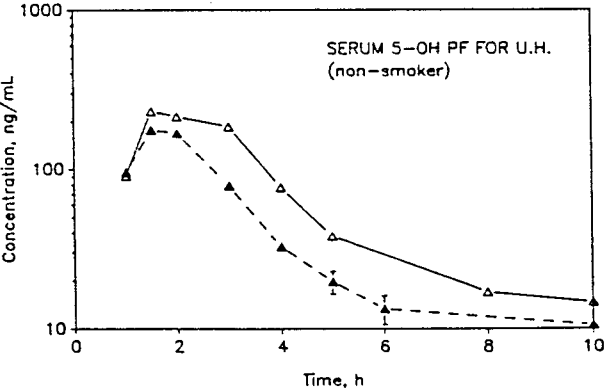
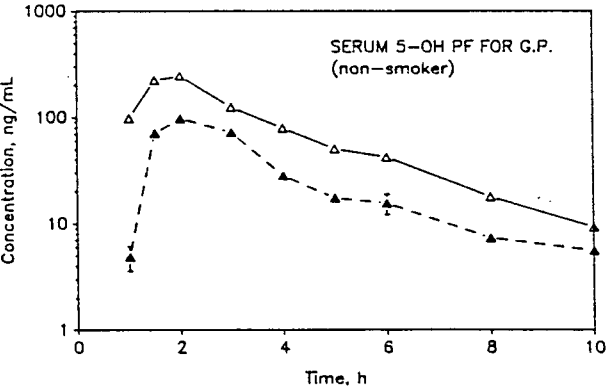
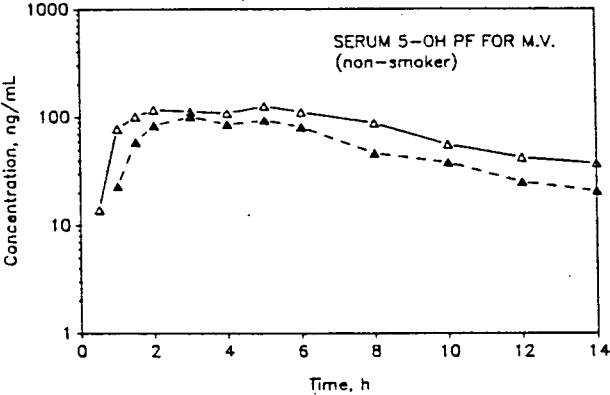
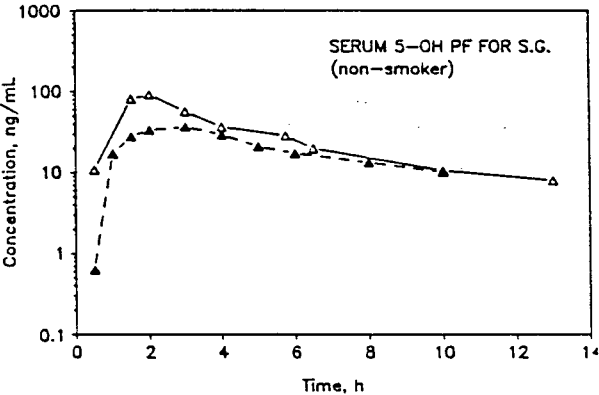
Appendix 3. (Cont'd)



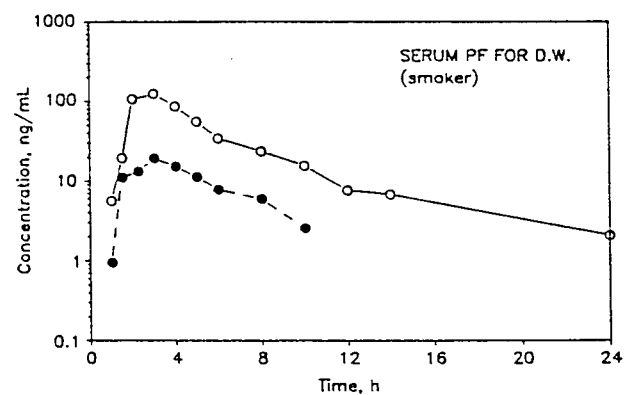
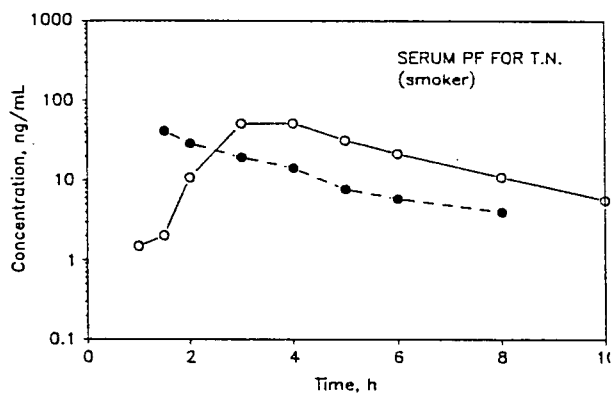
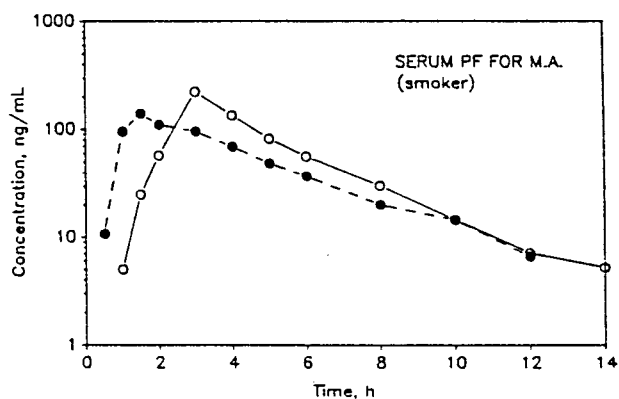
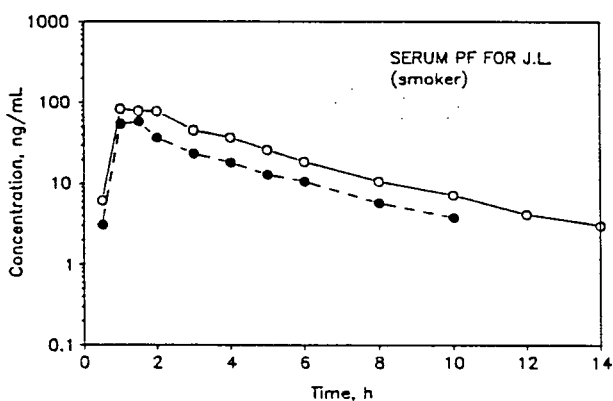
Appendix 4. Semi-logarithmic plots of the 5-hydroxy propafenone serum concentration *versus* time curves of eight non-smoking subjects before (Δ) and after 23 days of phenobarbital treatment (\blacktriangle).



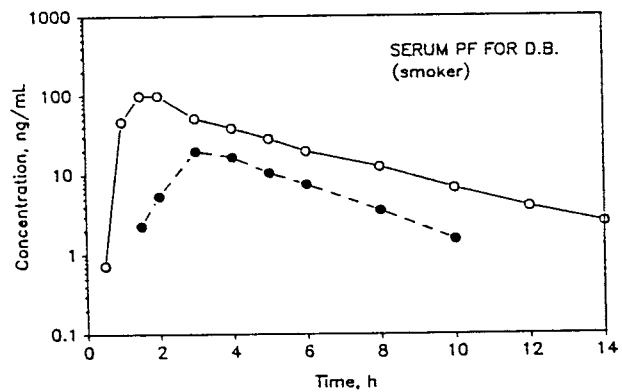
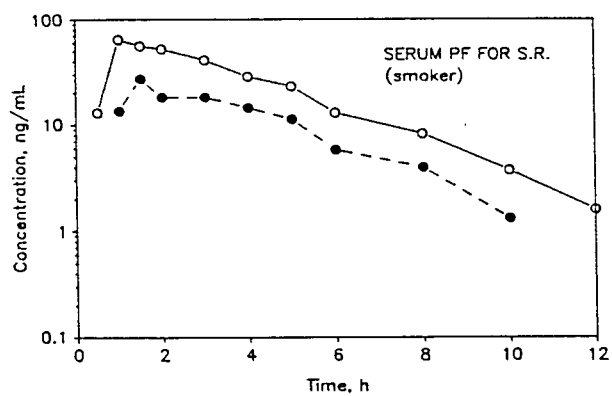
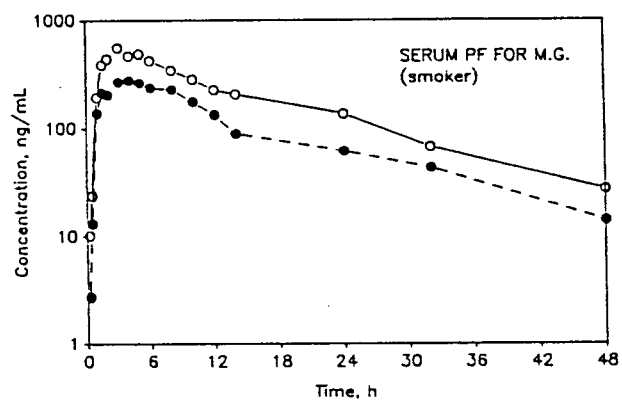
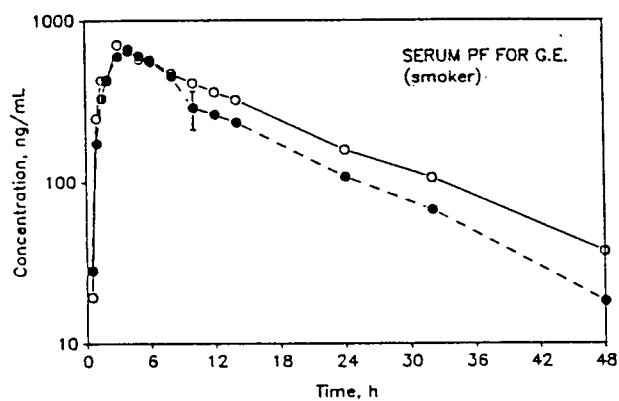
Appendix 4. (Cont'd)



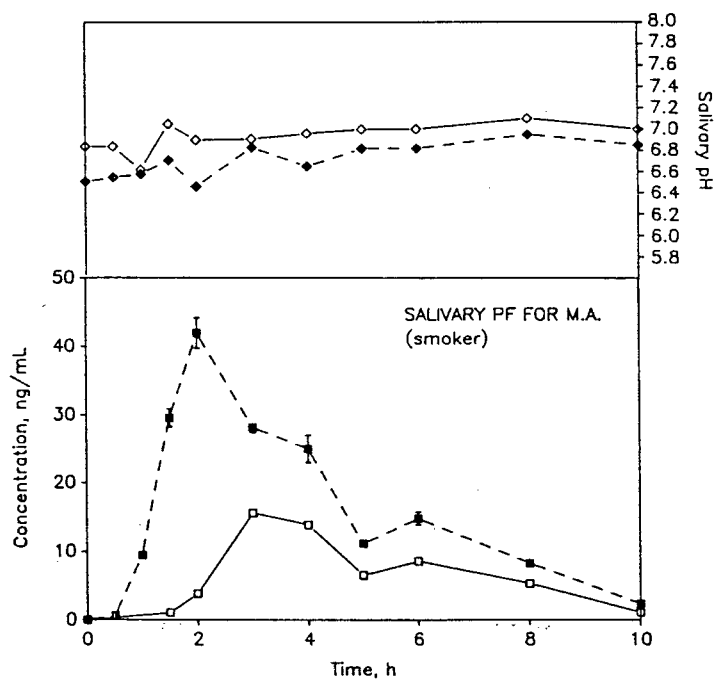
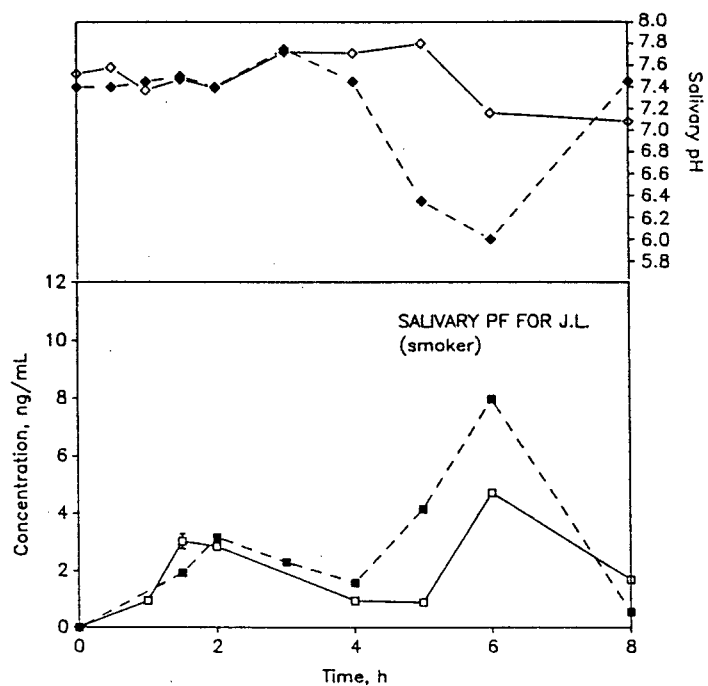
Appendix 5. Semi-logarithmic plots of the propafenone serum concentration versus time curves of eight smoking subjects before (○) and after 23 days of phenobarbital treatment (●).



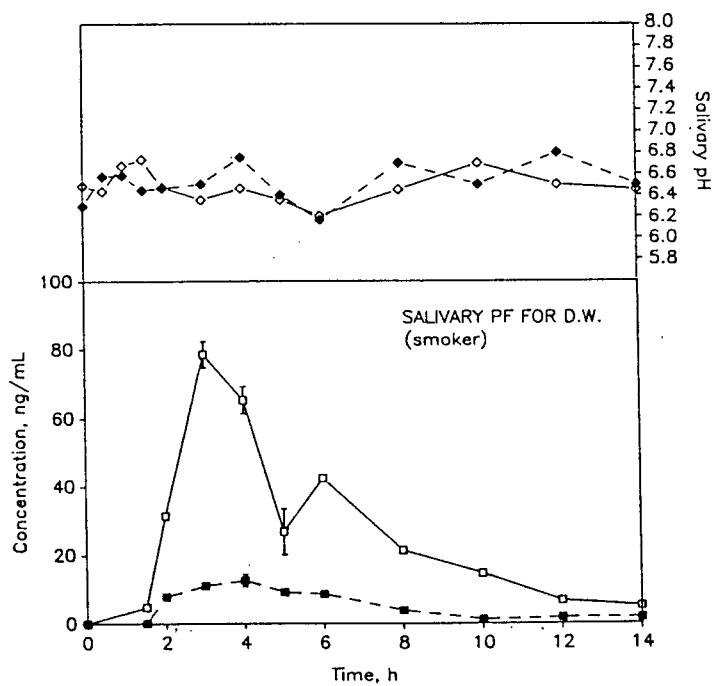
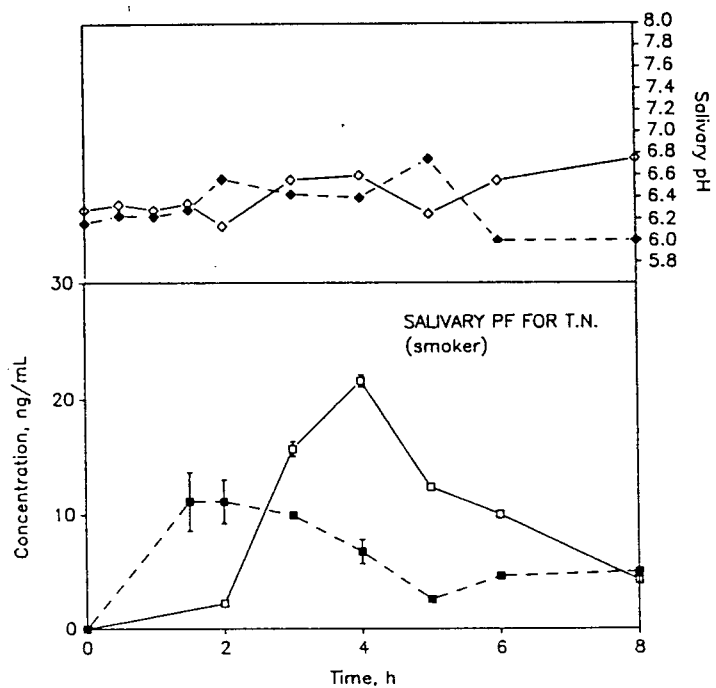
Appendix 5. (Cont'd)



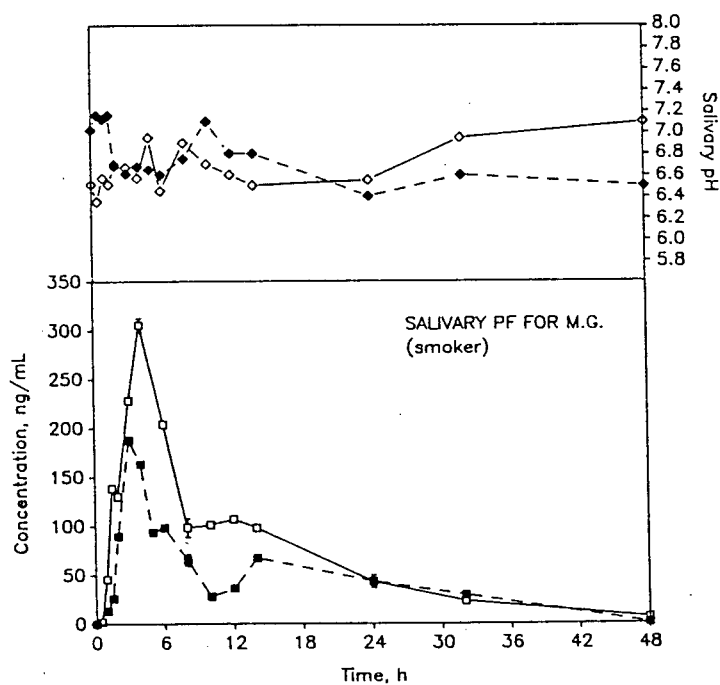
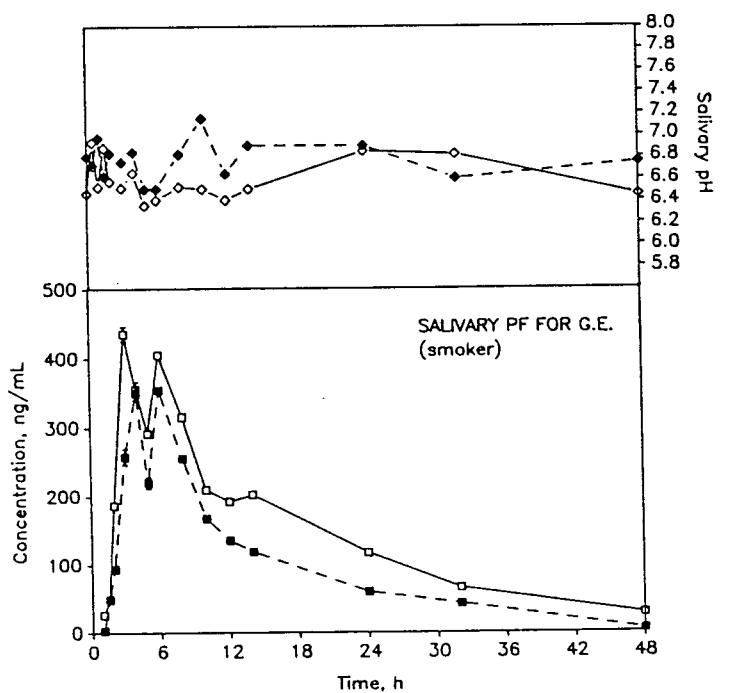
Appendix 6. Semi-logarithmic plots of the propafenone salivary concentration versus time curves of eight smoking subjects before (□) and after 23 days of phenobarbital treatment (■). Also shown in the figures is the pH of saliva before (◇) and after (◆) phenobarbital treatment.



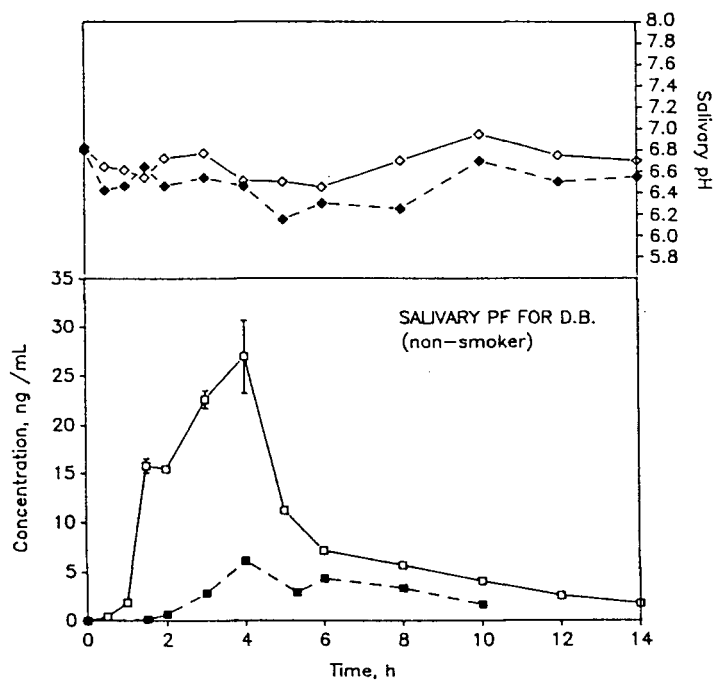
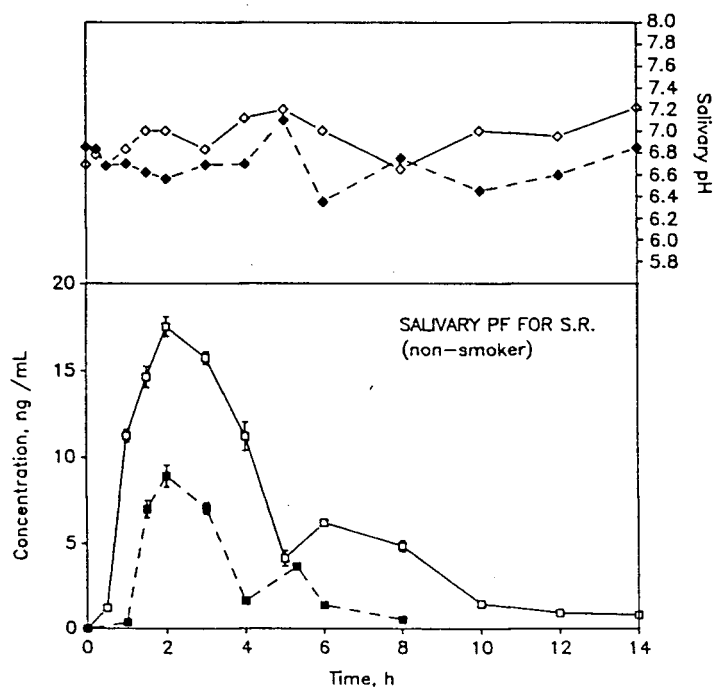
Appendix 6. (Cont'd)



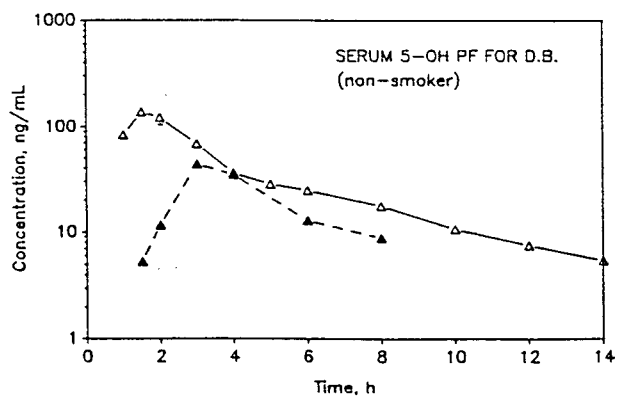
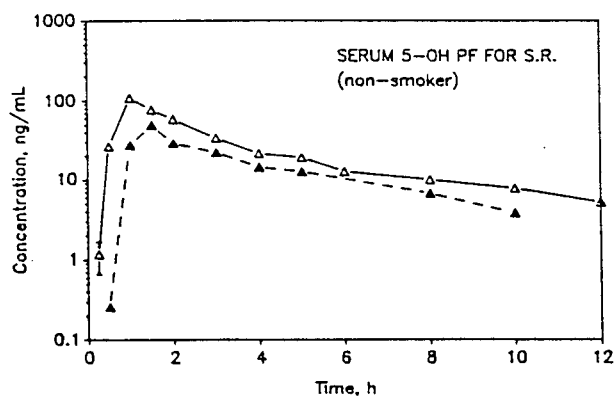
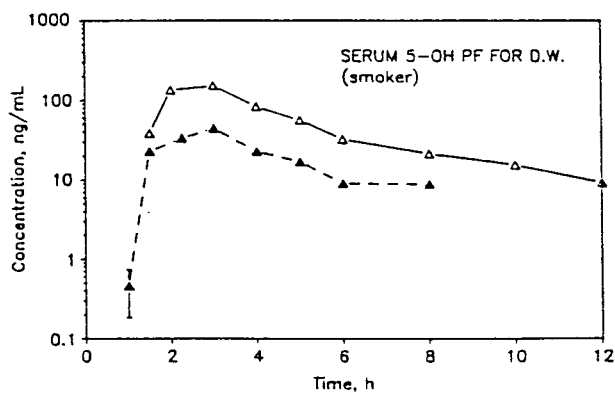
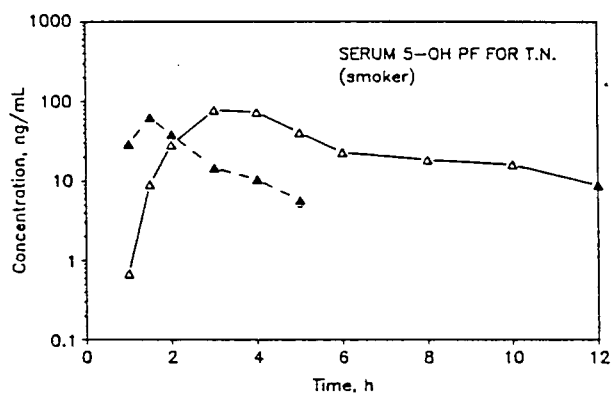
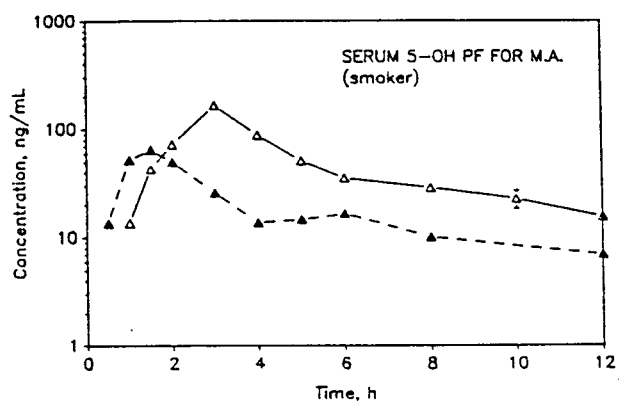
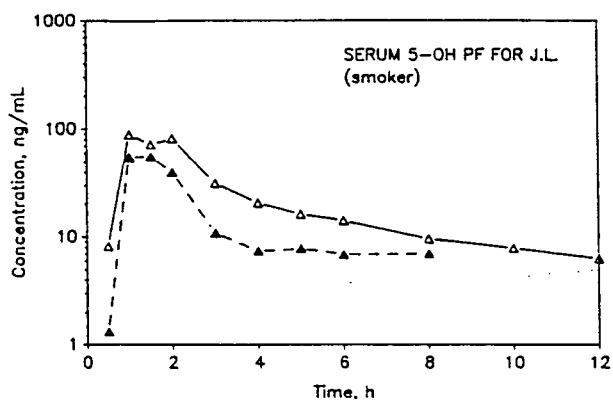
Appendix 6. (Cont'd)



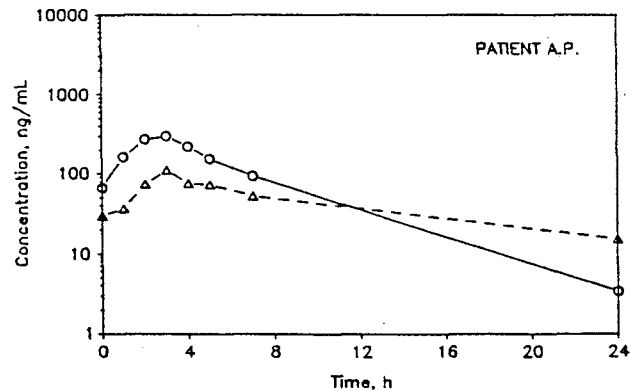
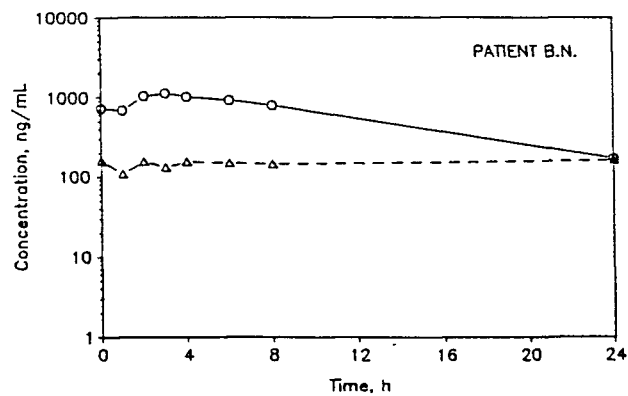
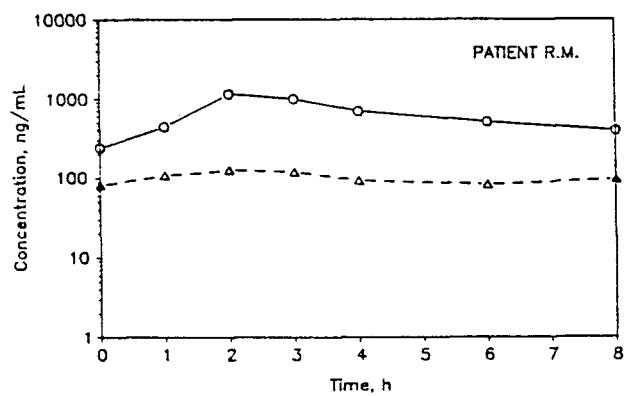
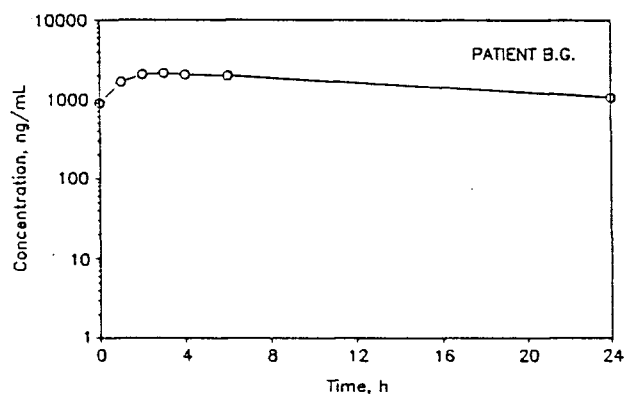
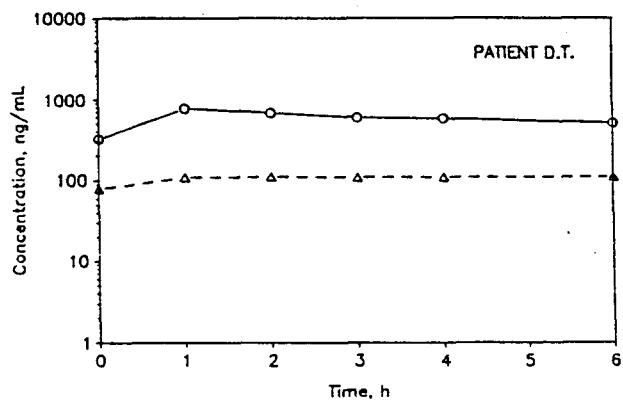
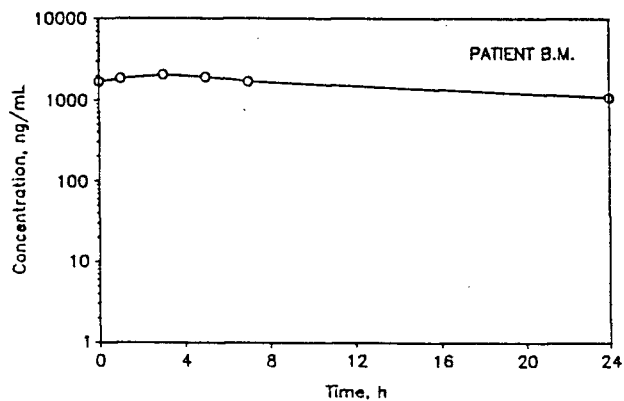
Appendix 6. (Cont'd)



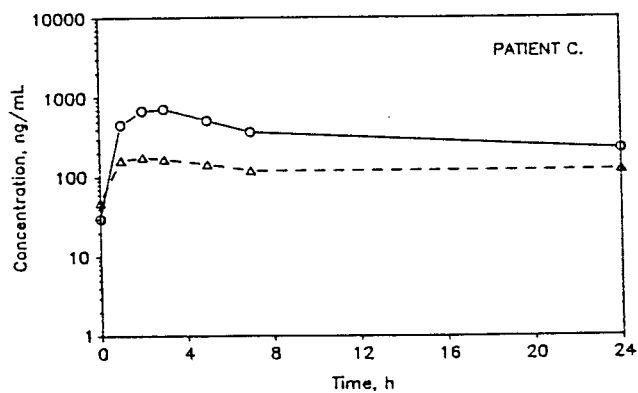
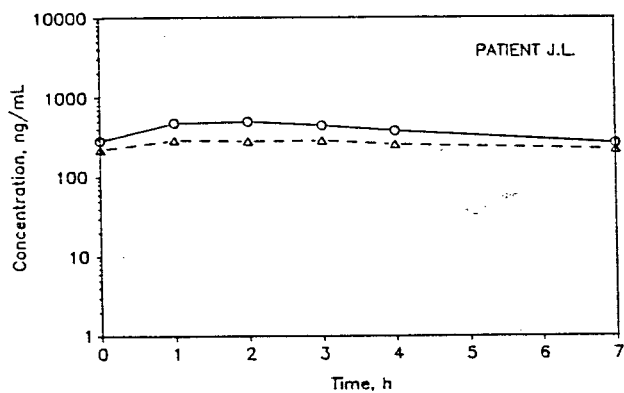
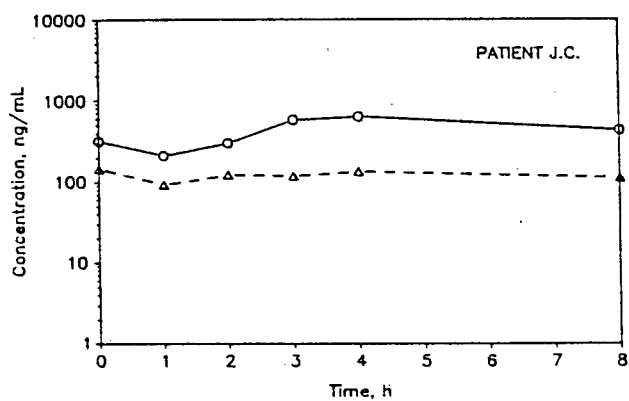
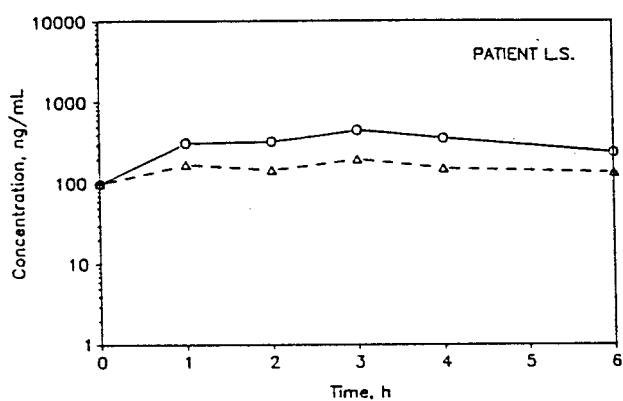
Appendix 7. Semi-logarithmic plots of the 5-hydroxy propafenone serum concentration *versus* time curves of six smoking subjects before (Δ) and after 23 days of phenobarbital treatment (\blacktriangle).



Appendix 8. Semi-logarithmic plots of the propafenone (O) and 5-hydroxy propafenone (Δ) serum concentration versus time curves of ten patients.



Appendix 8. (Cont'd)



Publications:

1. Wallace SM and Chan GL-Y: *In Vitro* Interaction of Aminoglycosides with β -Lactam Penicillins, *Antimicrob. Agents Chemother.* 28(2): 274-281, 1985.
2. Axelson JE, Chan GL-Y, Kirsten ED, Mason WD, Lanman RC and Kerr CR: Food Increases the Bioavailability of Propafenone, *Br. J. Clin. Pharmac.* 23:735-741, 1987.
3. Chan GL-Y, Axelson JE, Abbott FS, Kerr CR, McErlane KM: Determination of Propafenone in Biological Fluids by Fused-Silica Capillary Gas Chromatography Using Electron-Capture Detection, *J. Chromatogr. (Biomed. Appl.)* 417:295-308, 1987.
4. Chan GL-Y, Axelson JE, Price JDE, McErlane KM and Kerr CR: *In Vitro* Serum Protein Binding of Propafenone in Normal and Uraemic Human Sera, *Eur. J. Clin. Pharmacol.*, 36:495-499, 1989.
5. Chan GL-Y, Axelson JE, Abbott FS, McErlane KM and Kerr CR: Determination of 5-Hydroxy Propafenone, an Active Metabolite of Propafenone in Biological Fluids by Fused-Silica Capillary Gas Chromatography Using Electron-Capture Detection, (Letter to the Editor), *J. Chromatogr.*, 1989, in press.
6. Chan GL-Y, Axelson JE, Yeung J, McErlane KM and Kerr CR: Propafenone Pharmacokinetics and Metabolism: Effect of Inducers on Clearance and Binding, to be submitted to *Br. J. Clin. Pharmac.*, 1989.