PHENYLPROPANOLAMINE: ANALYTICAL AND PHARMACOKINETIC STUDIES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

by

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ABSTRACT

Phenylpropanolamine (PPA), a synthetic sympathomimetic amine structurally related to ephedrine has been widely used over the past 40 years as a nasal decongestant and appetite suppressant. It has been the focus of much controversy concerning the efficacy of the drug in its use as an anorectic agent, and due to the side effects caused by the higher doses of PPA required for appetite suppression.

Although extensively used, there is little information concerning the determination of PPA in biological fluids and on the pharmacokinetics of this drug. An adaptation of a published high-performance liquid chromatographic (HPLC) assay for PPA in serum and urine using U.V. detection at 210 nm is presented. PPA was separated in the reversed-phase mode. The method has a limit of sensitivity of 5.0 ng/mL and 10.0 ng/mL in serum and urine respectively. Serum concentration data following a single 25 mg dose of phenylpropanolamine in human volunteers demonstrate the application of the analytical method for bioavailability and pharmacokinetic studies.

After the administration of 25 mg, 50 mg or 100 mg PPA.HCl solutions to 5 human volunteers, a dose proportionality study demonstrated that PPA appears to exhibit linear kinetics. Linear one body compartment kinetics were assumed and the Wagner-Nelson method used to transform *in vivo* serum data to absorption plots. The serum data were fitted to a model using nonlinear regression techniques to characterize the pharmacokinetic processes of PPA. The absorption of phenylpropanolamine appears to be discontinuous and the drug seems to favour a two body compartment model. The pharmacokinetic parameters obtained from a steady state study using multiple dosing of PPA.HCl solutions compared

with those found from previous studies after the administration of sustained-release formulations.

A plasma protein binding study using equilibrium dialysis demonstrated that PPA is not highly protein bound in the blood.

CHAPTER 1

INTRODUCTION

Phenylpropanolamine hydrochloride is a synthetic sympathomimetic drug, structurally and pharmacologically related to ephedrine and amphetamine. The drug is a more potent vasoconstrictor than ephedrine or amphetamine but is less active in its central stimulant effect and has less beta-activity (1). Phenylpropanolamine is used widely as the active component of over-the-counter (OTC) preparations for the symptomatic relief of rhinitis, sinusitis and cough. It is also commonly found in OTC appetite suppressants and as a component of legal stimulants (1).

The most commonly reported adverse reaction of phenylpropanolamine is a rise in blood pressure but other reactions such as psychotic disturbances have also occurred (2).

1.1 PHYSICOCHEMICAL PROPERTIES OF PHENYLPROPANOLAMINE (4)

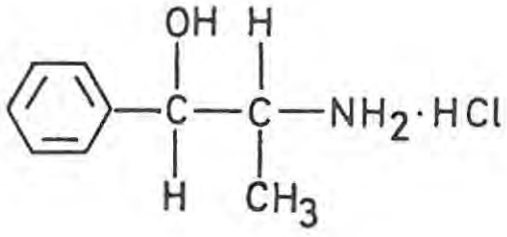
1.1.1 Description

1.1.1.1 Name, Formula, Molecular Mass

Phenylpropanolamine hydrochloride, sometimes referred to as dl-norephedrine, can also be named in a number of ways:

(a) α-(1-aminoethyl)benzenemethanol hydrochloride
(b) α-(1-aminoethyl)benzyl alcohol hydrochloride
(c) (±)-2-amino-phenylpropan-1-ol hydrochloride
(d) 2-amino-1-phenyl-1-propanol hydrochloride
(e) α-hydroxy-β-aminopropylbenzene hydrochloride
(f) 1-phenyl-2-amino-1-propanol hydrochloride

FIGURE 1.1 Phenylpropanolamine hydrochloride



$C_9H_{13}ClNO$ MM 187.67

1.1.1.2 Appearance, Odour, Colour and Taste

The compound is a white to creamy-white crystalline powder with an aromatic odour. It has a bitter taste (4).

1.1.2 Synthesis

Phenylpropanolamine hydrochloride is prepared by reacting benzaldehyde with nitroethane in 95% ethanol in the presence of sodium hydroxide to form α-(1-nitroethyl)benzyl alcohol and then reducing this nitro-alcohol to the corresponding amino compound. A stream of hydrogen chloride passed into a suitable solution of the base yields the hydrochloride (3).

1.1.3 Physical Properties

1.1.3.1 Solubility

Soluble in 2.5 parts of water and in 9 parts of alcohol (95 per cent); insoluble in chloroform and in solvent ether. After sonicating for one minute at ambient temperature the following solubilities were established (4).

TABLE 1.1 Solubility of PPA.HCl

Solvent	mg/ml	Solubility
Water	>50-<1 000	Soluble
Methanol	>50-<1 000	Soluble
Isopropanol	>10-<33.3	Sparingly soluble
Diethylether	>0.5	Practically insoluble
Ethyl acetate	>0.5	Practically insoluble
Chloroform	>0.5	Practically insoluble
Benzene	>0.5	Practically insoluble
Carbon tetrachloride	>0.5	Practically insoluble
Acetonitrile	>0.5	Practically insoluble
Acetone	>0.5	Practically insoluble
Cyclohexane	>0.5	Practically insoluble

1.1.3.2 Melting Range

Phenylpropanolamine hydrochloride crystals melt at 190-194°C. The free base melts at 101-101.5°C (5).

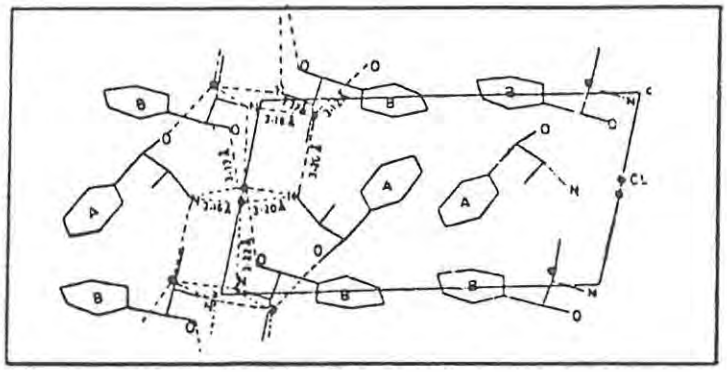
1.1.3.3 Specific Rotation

The specific rotation $[\alpha]^{25}_{D}$, of phenylpropanolamine hydrochloride in water is +32° (6).

1.1.3.4 Crystal Structure

Phenylpropanolamine hydrochloride has a monoclinic crystal system with possible space groups of $P2_1$ (non-centrosymmetric) or P2/m (centrosymmetric). The cell dimensions are a = 7.448Å, b = 9.461Å, c = 14.595Å, = 103.4° with each asymmetric unit containing two molecules (7). The crystal structure of phenylpropanolamine is shown in Fig. 1.2.

FIGURE 1.2 The crystal structure projected along y. Solid circles denote chloride ions



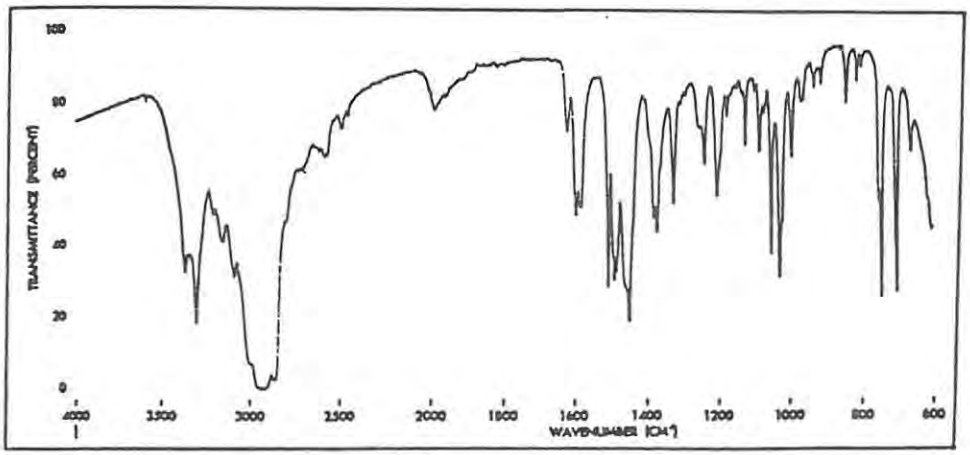
1.1.3.5 Dissociation Constant

The pK_a of phenylpropanolamine hydrochloride determined potentiometrically at 20°C is 9.44 $\pm$ 0.04 (8).

1.1.3.6 Infrared Spectrum

The infrared spectrum of phenylpropanolamine hydrochloride is shown in Fig. 1.3. It was obtained from a Nujol mull between KBr plates.

FIGURE 1.3 Infrared spectrum of phenylpropanolamine hydrochloride in Nujol mull.



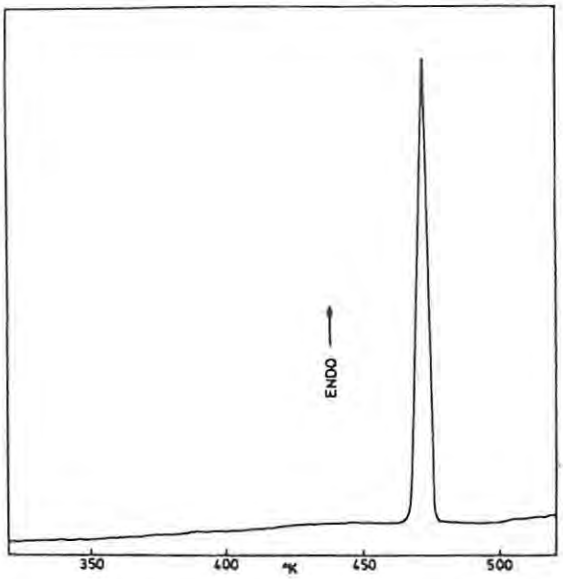
Characteristic band assignments are listed below (4).

Frequency (cm^{-1})	Assignment
3368	O-H stretching
3303	NH_3^+ stretching
2800-2400	NH_3^+
1990	NH_3^+
1623	NH_3^+ out of plane deformation
1598	C=C aromatic stretching
1581	NH_3^+ out of plane deformation
1508, 1491	C=C aromatic stretching
1450	O-H out of plane deformation
1329	NH_3^+ in plane deformation
1241, 1208	O-H in plane deformation
1128,1088, 1054	C-H in plane deformation, monosubstituted benzene
1031	C-O stretching
816, 802	NH_3^+ rocking
747, 703	C-H out of plane deformation, monosubstituted benzene

1.1.3.7 Differential Scanning Calorimetry

A single endotherm was observed with an onset temperature of 194.5°C which corresponds to the melting point. The heat of transition (ΔH melting) calculated in relation to an indium standard is 168 $\pm$ 6 Jg^{-1}. The thermogram is depicted in Fig. 1.4 (4).

FIGURE 1.4 Differential scanning calorimetry curve of phenylpropanolamine hydrochloride.

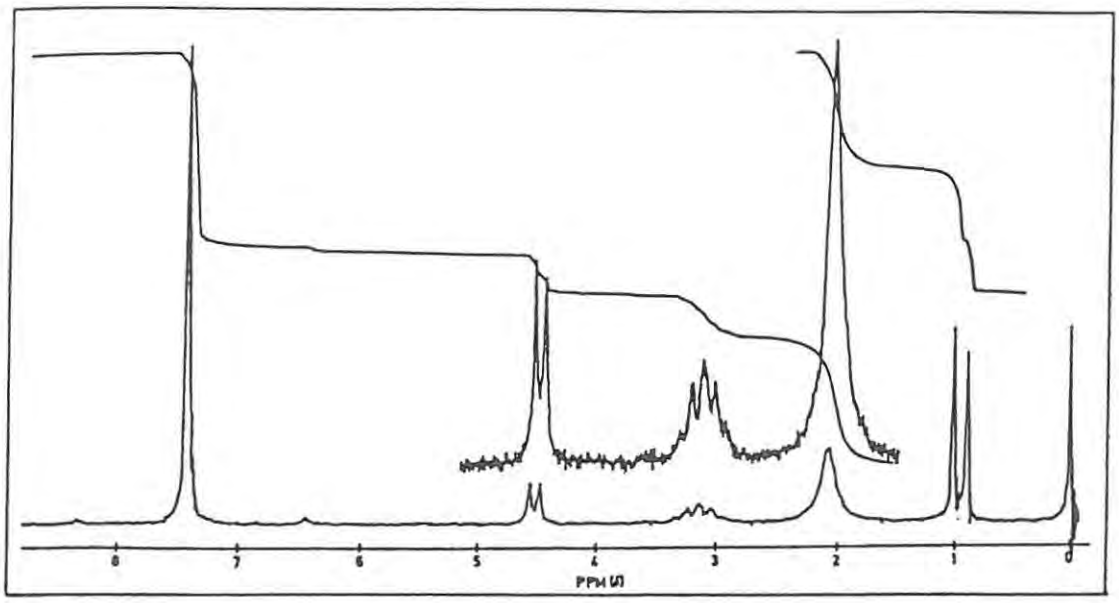


1.1.3.8 Proton Magnetic Resonance Spectrum

The 60MHz proton magnetic spectrum of phenylpropanolamine base in CDCL3 with tetramethylsilane (TMS) as the internal standard is depicted in Fig. 1.5 (4). The integration and multiplicities are consistent with the proton assignments. Chemical shifts (δ) in ppm relative to TMS are:

Proton assignment	Number of protons	J(Hz)	Chemical Shift (δ)	Multiplicity
$-CH_3$	3	9.0	0.94	Doublet
$-NH_2$, -OH	3	-	2.07	Singlet
N-C-H	1	9.0	3.12	Quintuplet
O-C-H	1	9.0	4.51	Doublet
Aromatic	5	-	7.41	Singlet

FIGURE 1.5 Proton magnetic resonance spectrum of phenylpropanolamine in $CDCl_3$



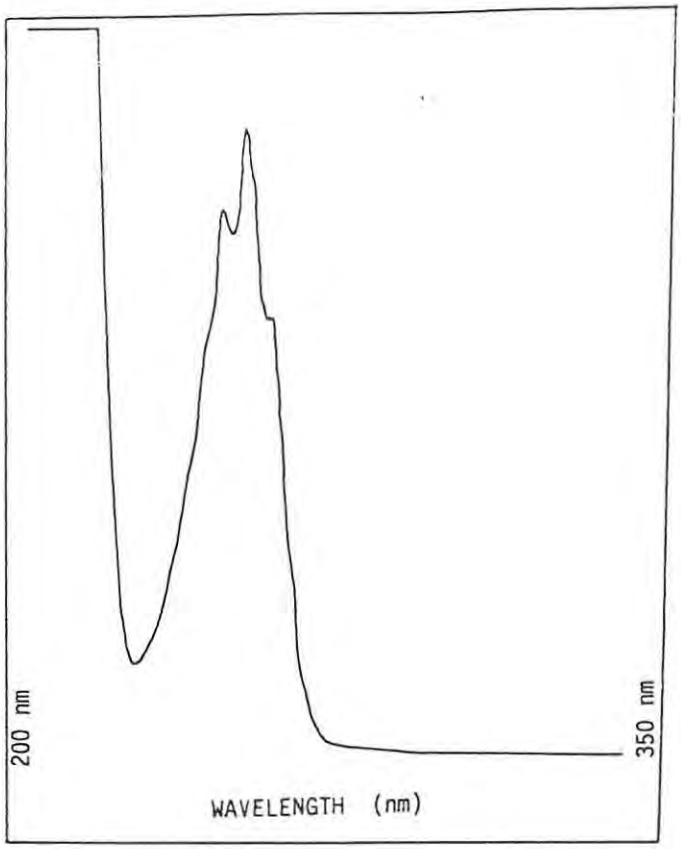
An inspection of the D_2O exchange spectrum shows disappearance of the resonance at 2.07 ppm. This corresponds to three protons, one hydroxyl proton and two amino protons.

1.1.3.9 Ultraviolet Spectrum

The ultraviolet spectrum of phenylpropanolamine hydrochloride in methanol at a concentration of 1 mg/ml was obtained with a Beckman Acta M VI ultraviolet spectrometer. The spectrum obtained in methanol is depicted in Fig. 1.6. and shows shoulders at 267, 248 and 243 nm.

Solution	Absorption Maxima (nm)	E
Methanol	264.0	133.63
	258.0	179.04
	252.0	145.44

FIGURE 1.6 Ultraviolet spectrum of phenylpropanolamine hydrochloride in methanol.

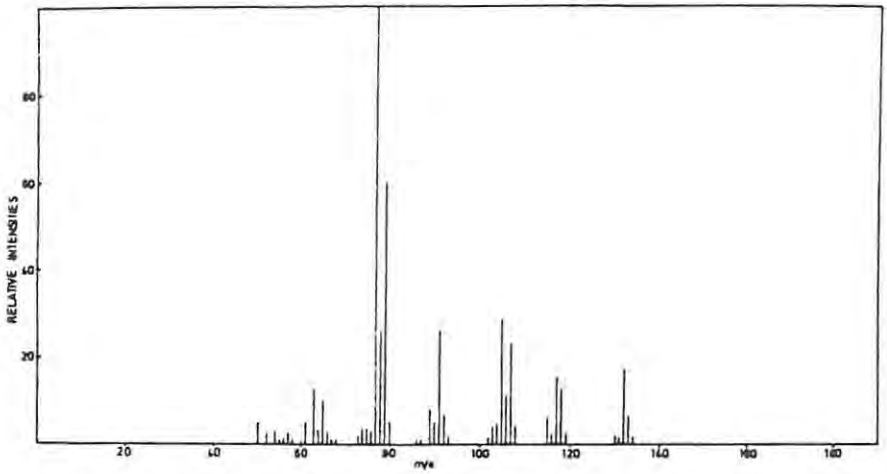


1.1.3.10 Mass Spectrum

The low resolution mass spectrum of phenylpropanolamine hydrochloride is shown in Fig. 1.7. Direct probe at 80°C into the ion source was used to obtain the mass spectrum (4). The molecular ion is not observed. The assignments of some of the major ions formed are:

m/e	%	Ion
132	17	$Ph-CH=C(CH_2)NH_2^+$
105	29	$Ph-C=O^+$
91	26	$Ph-CH_2^+$
77	100	Ph^+

FIGURE 1.7 Mass spectrum of phenylpropanolamine hydrochloride.



1.2 MODE OF ACTION OF PHENYLPROPANOLAMINE

Phenylpropanolamine hydrochloride (PPA. HCl) is a sympathomimetic phenylethylisopropylamine with largely indirect actions. It displaces endogenous sympathetic transmitters particularly norepinephrine from storage sites. PPA has some direct effects (9, 10). It posesses both alpha-1 and beta-1 agonist properties in vitro (9, 11), and minor central nervous activating effects. The alpha effects are predominantly vasoconstrictive in character although bladder trigone and uterine smooth muscle may be effected. The beta effects are occasionally seen as increased heart rate although tachycardia is seldom noted in animals or humans with intact cholinergic reflex mechanisms (12). It is not clear from published data whether PPA has beta-2 agonist activity.

An indirectly-acting sympathomimetic amine crosses the cell membrane of adrenergic neurones, usually by an active process. Within the axon varicosity, it crosses the membranes of storage vesicles and displaces noradrenaline in the cytoplasm which may then act on the effector cell (13) or be O-methylated by an enzyme, catechol-O-methyltransferase (COMT). The responses elicited are therefore similar to those of noradrenaline (13). Indirectly-acting sympathomimetics also exhibit tachyphylaxis; that is, repeated injections of these drugs become less effective as the releasable stores of noradrenaline are depleted (13).

Phenylpropanolamine primarily causes the arterioles to contract by alpha- adrenergic stimulation, and only slightly influences capillary width (14). It is believed that PPA constricts the pre-capillary sphincters by its direct action on alpha-adrenergic receptors, and thus reduces blood flow to the turbinates thereby minimizing venous pooling with its resultant nasal congestion (15).

The central nervous system (CNS) actions of PPA are not completely understood (16). PPA is absorbed into the blood after oral administration, crosses the blood-brain barrier and acts as a CNS stimulant via its sympathomimetic activity (17). This activity is related primarily to the blockage of presynaptic neuronal uptake of catecholamines, introduction of an increased release of noradrenaline from the presynaptic neuron, monamine oxidase inhibition, and agonist stimulation of post-synaptic alpha receptors (18).

Phenylpropanolamine has some central stimulating effects, manifest as improved reaction skills and flicker recognition (19, 20). The basic stimulatory action of sympathomimetic drugs is probably to increase arousal via noradrenergic pathways in the ascending reticular activating system (13).

The neurophysiological substrate of appetite suppression is not known. Previous theories of hypothalamic centres that subserve appetite and satiety are no longer tenable, and the newer ideas that relate appetite to specific neurotransmitters have not enabled clear statements to be made about drug action (21). However, it can be stated broadly that PPA seems to act in a manner that is pharmacologically similar to amphetamine, which probably involves the release of adrenaline or noradrenaline in the prefornical region of the brain (22). The anorectic and motor activity actions of PPA have been characterized by Cairns *et al.* (23) in which a catecholaminergic component in PPA action has been confirmed.

Phenylpropanolamine produces weight loss that may result, in part, from an excitatory action of PPA on brown adipose tissue (BAT) thermogenesis (24), which is indicated primarily via activation of beta-adrenergic receptors. The absence of an effect of beta-adrenergic blockade on PPA anorexia supports this suggestion (25). The thermogenic action of PPA may be attributed to the vasoconstrictive action of PPA that would limit the loss of heat through the skin (24).

The discovery of brain peptides as neurotransmitters opens a new chapter in the study of feeding from a neural point of view. Current studies suggesting a limbic system framework of chemical neuroanatomy in which peptides play an important role in the control of eating have been reviewed (25). In this framework, anorectic drugs such as PPA act on dopamine, norepinephrine and serotonin systems that ascend from the hindbrain and midbrain to modulate feeding and satiety systems in the hypothalamus (26).

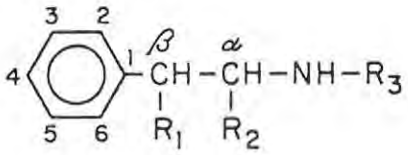
The glucostatic theory of feeding control suggests that anorexia could be caused by a drug with an insulin-like action, or a direct excitatory action on glucoreceptors (16). A study by Resnick *et al.* (27) investigated the hypoglycaemic effects of intraperitoneal PPA on rats to test the possibilities that it might derive some of its anorectic properties through effects on glucostatic mechanisms. It was found that PPA tended to lower blood glucose levels in normal and diabetic rats; this is the first evidence that PPA might possibly reduce feeding by increasing blood sugar utilization.

1.2.1 Structure - Activity Relationships of Phenylpropanolamine

Agents that lack hydroxyl groups on the aromatic ring, i.e. non-catecholamine derivatives, can exhibit alpha and beta effects, mainly by indirect mechanisms (13). Substitution on the alpha-carbon atom blocks oxidation by monamine oxidase (MAO), thus greatly prolonging the duration of action on non-catecholamines, the detoxification of which depends largely on breakdown by MAO since they are unaffected by catechol-O-methyltransferase (COMT). Compounds with an alpha-methyl substituent persist in the nerve terminal and are more likely to

release noradrenaline from storage sites. In general, then less substitution in the amino group, the greater is the selectivity for alpha-activity.

FIGURE 1.8 Sites of potential structural variance of sympathomimetic phenylethylamines



Substitution of an hydroxyl group on the beta-carbon generally decreases central nervous stimulant (CNS) action, but enhances peripheral organistic activity, both at alpha and beta receptors. Hydroxyl substitution increases polarity and dramatically reduces lipid solubility. For example, the chloroform/water partition coefficients for dextroamphetamine, ephedrine and PPA are 146, 0.015 and 0.001, respectively (28). Beta-hydroxyl-substituted products are considered to be indirect agonists (13, 29, 30), that act by enhancing the release of stored catecholamines and/or by inhibiting catecholamine uptake (31).

1.3 PHARMACOKINETICS OF PHEYLPROPANOLAMINE

1.3.1 Absorption and Distribution

After oral administration of phenylpropanolamine hydrochloride as an aqueous solution, absorption is rapid, being completed in less than 2.5 hours (4). However, absorption can be altered by a variety of factors, particularly the presence of absorbents such as activated charcoal (32). Phenylpropanolamine, being a weak base will not be absorbed readily from the acidic stomach due to ionization. In the

alkaline intestine absorption does occur at rates determined by the dissociation constant and the lipid solubility of the unionized form. Although the pKa of PPA is 9.4, it is almost completely absorbed in the small intestine due to the large surface area available for absorption (8).

The tissue distribution of the drug in dogs 2 hours after administration is kidney > lung > liver > spleen > brain > heart > muscle > plasma > fat > cerebrospinal fluid (4). The presence of a beta-carbon hydroxyl group establishes an active site which tends to dissociate in solution, markedly increasing the polarity and water solubility of PPA (33). Water soluble, metabolically stable forms (refer to section 1.3.2) poorly penetrate cellular barriers to reach sites of further metabolism, or sites of action in the central nervous system. From recent studies, PPA has been found to have an apparent volume of distribution of 300 litres (34).

1.3.2 Metabolism

Relatively little information has been reported on the metabolism of phenylpropanolamine hydrochloride. The drug is referred to by Axelrod as a "metabolically stable entity" (35). In urine studies with human subjects it was found that approximately 90% of the drug was excreted in 24 hours, predominantly unchanged. Sinsheimer *et al.* (36) found only 4% as transformation products, the major biotransformations being parahydroxylation to 4-hydroxynorephedrine and oxidative deamination of the side chain, resulting in hippuric acid. Phenylpropanolamine is also metabolised by phenylethanolamine-N-methyltransferase to the corresponding N-methylated metabolite. In the rat and rabbit, 80-90% of ^{14}C of small doses of ^{14}C norephedrine is excreted in the urine within 24 hours, with 1-2% of the dose being excreted in the faeces. In the rat, 60% occurs as the unchanged drug and 35% as 4-hydroxynorephedrine. Small amounts of 4-hydroxyhippuric acid and 1,2-dihydroxy-1-phenylpropane were also detected. In the rabbit, however, 76% is excreted as deamination products consisting of 31% 1,2-dihydroxy-1-phenylpropane, 27% 1-hydroxy-2-oxo-1-phenylpropane and 24% benzoic acid (4).

Phenylpropanolamine appears as a minor metabolite of amphetamine, methamphetamine (37), ephedrine (38) and as a major metabolite of cathinone (39).

1.3.3 Excretion

The mean elimination half-life of phenylpropanolamine hydrochloride in man has been reported as 3.9 hours and the elimination rate constant as 0.18 hr^{-1} (40). In a study with human subjects under controlled conditions of urinary pH, acidification of the urine resulted in the excretion of largely unchanged PPA.HCl, whereas basic urine conditions resulted in reabsorption of the drug with little influence on metabolism (41). Excretion is generally delayed if the urine is alkaline. Since the pKa of PPA is greater than 8.5, excretion of the unchanged form is generally increased when the partition between acidic urine and more alkaline body fluids would favour diffusion into the acidic urine (42). PPA is excreted by filtration at the glomerulus (43).

1.4 USES OF PHENYLPROPANOLAMINE

First synthesized in 1910 (4), PPA has been pharmacologically and clinically evaluated repeatedly since its identification as a potentially useful sympathomimetic. Phenylpropanolamine's initial introduction into medicine was as a parenterally administered compound for the maintenance of blood pressure in postoperative hypotensive patients with significantly fewer side-effects than ephedrine. However, as a pressor medication, PPA was superseded by more effective agents and is no longer used for this purpose.

Phenylpropanolamine is found in four types of products:
(a) prescription (b) over the counter (OTC) cough-cold and allergy preparations, (c) OTC appetite suppressants and (d) "Look-alike" combinations, where it has been abused as a substitute for amphetamine (the availability of which has been diminished by recent federal and state regulatory actions in the United States of America) (44).

Phenylpropanolamine is used mainly as a nasal decongestant for the treatment of colds and sinusitis, and for symptomatic treatment of allergic disorders due to hayfever, asthma, allergic rhinitis, urticaria, angioneurotic oedema and bronchospastic conditions accompanying allergic asthma (45). The drug is often used combined with analgesics, antihistamines, anticholinergics, caffeine, atropine-like drugs and other products to diminish the discomforts of upper respiratory conditions (46). PPA can be found in many oral formulations including tablets, capsules, syrups, elixirs and suspensions (47), and is administered in doses of 12.5 to 50 mg every 3 to 8 hours. Oral administration can reach areas inaccessible to topical applications and may provide a longer duration of action. Systemic use, however, may be complicated by undesirable sympathomimetic effects (48).

Decongestion of mucous through vasoconstriction may be achieved by local application of PPA, which acts directly on alpha-receptors. Phenylpropanolamine is used in concentrations varying from 1% to 3% in nasal solutions and jellies (49). Topical administration has the advantage of more rapid onset, but may not reach all areas of the nasal mucosa. Also, the topical route exposes the patient to the possibility of the so-called rebound phenomenon in which the nasal mucous membranes become even more congested as the drug's vasoconstrictor effect wears off, encouraging the overuse of the product (50).

Another action of PPA consists of a moderate inhibition of mucosal secretion, and an anti-allergic effect (51, 52). Phenylpropanolamine has a subtle but measurable anti-congestive effect (53), and is found to be useful in improving air flow in the nasal passages (54). Phenylpropanolamine is often used in combination with antihistamines to control nasal congestion and to counteract partially the drowsiness produced by antihistamines (19). Decongestant-antihistamine mixtures containing PPA in the prevention and treatment of otitis media with effusion in children have been found to be clinically useful (55).

Sporadic reports have demonstrated the usefulness of sympathomimetic agents such as PPA in correcting retrograde ejaculation due to neurogenic dysfunction (56-58) PPA could be of benefit in establishing antegrade ejaculation even in the absence of neurogenic dysfunction (59). Phenylpropanolamine has recently been indicated to ameliorate symptoms in patients suffering from orthostatic hypotension after general measures of treatment have failed. Although doses of 25 mg can have a significant pressor effect, doses of 75 mg may be required with multiple system atrophy (60). It has been reported that PPA taken at bedtime is of some benefit for adults who suffer from mild or occasional snoring, the lay term for obstructive breathing during sleep (61). In combination with paracetamol and phenyltoxamine citrate, PPA has been reported to be of some benefit in cluster headache (Harton's headache) (62).

Bladder storage may be improved by increasing bladder outlet tone at the proximal portion of the urethra with alpha-stimulating agents (63, 64), and PPA in a dosage of 50 mg twice daily seems to be of some use in relieving stress during incontinence. Schreiter *et al.* (65) support the idea that PPA elevates the urethral pressure more effectively when administered in combination with oestrogens, especially when used in elderly female patients with urinary incontinence.

In a recent report, PPA has been found to be useful in counteracting and antagonizing several effects of diazepam, mainly unwanted ones, without decreasing the calming effect of diazepam (20).

Clinically, PPA.HCl has been used as an anorexiant for over 40 years, following the appearance of an article by Hirsch (66), in which he concluded that PPA was effective in controlling appetite of patients on an anti-obesity diet. While it has been subject to both debate and dispute when used as an adjunct to control obesity, a spate of studies in recent years have served to substantiate its efficacy as well as its safety for this use (45, 67-69). In more recent clinical studies, comparing the efficacy of PPA in tablet form against a placebo control

or against a reference prescription appetite suppressant, PPA was significantly superior in achieving weight loss and equally effective as the prescription drugs, but with fewer side-effects reported by the PPA groups (70, 71). In a systematic study on mice, Cairns *et al.* (72) investigated the effects of PPA and sympathomimetic agents on food consumption. All anorectic agents caused a significant fall in food intake during the first hour after ingestion and thereafter there was a variable increase in feeding. In animal studies, weight change or food consumption is measured against an index of appetite. However, many effects of anorexiants, such as sedation, nausea, dyskinesia can cause reduced food intake in animals but have no relevance to appetite and satiation. In view of known differences in disposition of drugs in different species, a dose correlation as found in animal studies (73) should be considered a coincidence rather than a reflection of similarity.

In a recent summary Weintraub (1985) (74) reviewed a number of PPA clinical trials, published and unpublished. His criticisms went chiefly to the designs constructed to show efficacy, and did not cite evidence of bias regarding safety data.

Appetite suppressant products available in the United States include Dietac™ (50 mg) Appedrine (25 mg), Dexatrim Extra Strength™ (75 mg) and Panamine™ (37.5 mg). The smaller dose products are usually sold in immediate release formulations, while those containing higher doses are available only as sustained-release preparations. Recommended daily dosages vary, but none exceed 75 mg. Package instructions usually specify that the products should not be taken for more than 3 consecutive months.

Two firms have reached the letter-of-intent stage for development of a once-a-day nonprescription controlled-release transdermal delivery system described as a "molecular sponge" that has the mechanical properties of plastic. One of the first ingredients being considered for the new delivery system is the diet aid PPA (75).

A recent study evaluating the anorectic activity and safety of PPA.HCl (25 mg) in the form of liquid drops, concludes that PPA is safe and effective as an appetite suppressant in the treatment of exogenous obesity (76). For those patients who find it difficult to take capsules or tablets, PPA drops may be a comfortable alternative.

In conclusion, well-conducted clinical studies have repeatedly shown PPA to be safe and effective as a nasal decongestant and anorexiant. That PPA lacks significant potential for abuse or habituation has been demonstrated in a series of animal and human studies (19,77). Phenylpropanolamine began to appear in "street stimulants" in the late 1970's (78). The street drug "look-alikes" were not controlled by prescription, and contained ingredients including caffeine, ephedrine, pseudoephedrine and PPA, alone or in combination. Their sale, however, was banned in 1983 in the United States of America.

1.5 SIDE EFFECTS OF PHENYPROPANOLAMINE

In assessing adverse drug reactions, it is often difficult to identify the contribution of a specific substance, in a context of drug interactions, pre-existing disease conditions, the possibility of overdose, and the hazards of accepting patient identification of drugs without verification of their presence by analysis of body fluids.

For many years the medical literature has contained reports of adverse reactions to products which contain PPA. The most commonly reported reaction is a rise in blood pressure. There is no doubt that PPA in sufficiently large doses is capable of both alpha- and beta-agonist action, with a potential for causing hypertension, tachycardia, headaches, arrhythmias and encephalopathy.

Evidence from reported cases confirms that ingestion of PPA may sometimes be associated with severe blood pressure increases in previously normotensive patients (2, 79-81), and in patients with special sensitivity to adrenergic pressor agents (82, 83). There is also a strong suggestion that a dose-response relationship exists (2,

84, 85), with most cases of spontaneous hypertension occurring after ingestion of more than 50 mg PPA. Reports on hypertensive episodes, however, lose much of their impact, because these are often related to concurrently used drugs, diseases and flawed experimental design.

There have been a number of reports from Australia, Great Britain, Sweden and the United States of increases in blood pressure associated with PPA consumption. Although most of these cases have occurred after ingestion of higher than recommended doses of PPA, there have been sporadic reports of increased blood pressure after doses as low as 50 mg. The differences between reports of PPA-related hypertensive problems from European and Australian centres may be related to the pharmacologic effects resulting from the different stereoisomers of PPA which are marketed in these areas.

Additional evidence suggests that PPA-induced hypertension may result in cerebrovascular haemorrhage (86-88, 93), myocardial injury (89) and cardiac arrhythmias (90-94). A number of cases of adverse cardiovascular events following PPA ingestion have been reported (88, 93, 95). Some of these patients had taken overdoses of PPA in either cough-cold or diet-aid products (93, 95), while other case reports failed to specify the exact dose taken (88). That large doses of a sympathomimetic amine can produce hypertension, cardiac tachyarrythmias, headaches, encephalopathy, and even acute renal tubular or myocardial necrosis, should not be surprising in view of the known pharmacologic effects of PPA. Two young men consuming relatively large doses of diet-aid products for a period of time developed hemiparesis and evidence of cerebral infarction without evidence of cerebrovascular disease (96). The authors speculate that an "angiitis" perhaps associated with vascular spasm had occurred. Several adverse reactions were associated with a now unavailable version of the Australian product, Trimolets (82, 84, 97).

Phenylpropanolamine has been implicated in causing injury to the myocardium, as described by Pentel *et al.* (89). The authors reported 3 cases of increased blood pressure accompanied by rises in the levels

of serum creatine kinase and cardiac-iso-enzymes usually associated with myocardial infarction. Two patients had taken excessive doses and the third had taken a combination product. Excessive catecholamine stimulation in dogs can produce myocardial necrosis, which is thought to be an explanation for the reported adverse effect.

Since information is available about undesired cardiovascular responses and anorexiant doses of PPA animal studies of relative potencies are of little utility.

In a recent literature search, four published case reports (86, 88, 96, 98) cited a link between the ingestion of a PPA product and subsequent occurrence of stroke related symptoms. The relative paucity of reports linking stroke to PPA is noteworthy; none of these reports could prove or confirm any association between PPA and intra-cerebral haemorrhage. Hypertensive crises, and intra-cranial haemorrhages culminating in death have also been ascribed to PPA (86, 92, 95, 98). However, the vast majority of clinical situations involving look-alike stimulants have been associated with the high-dose use of combinations of PPA with caffeine and/or ephedrine.

There have been several case reports of psychiatric disorders related to the ingestion of nasal decongestants, anorexiant agents or "look alikes" containing PPA (alone or in combination) (99-109). Symptoms of psychotic episodes included auditory and visual hallucinations, confusion, disorientation, delusions, aggressive behaviour, impulsivity, mania, paranoia, agitation, anxiety, bizarre behaviour and depression. The most consistent characteristic shared by many of these patients is a positive psychiatric history. Phenylpropanolamine products probably exacerbated trait-related symptoms in patients with positive histories and caused acute drug-induced brain syndromes in patients with no psychiatric history. All cases required medical attention, but in cases where combination products were taken, especially those containing caffeine, it was observed that hospitalization was required.

Dietz (110) in 1981 reported seven cases, all emergency admissions to hospital, of severe side-effects occurring within a couple of hours of taking a single dose of a PPA-containing preparation (50 mg or 75 mg). The patients exhibited the same type of mental disturbance normally associated with the ingestion of amphetamine. Acute central nervous system effects also included hyperventilation, probably the result of stimulation of the medullary respiratory centre. Although this explanation is speculative, the reports indicate that patients with past histories of major or minor affective disorders are at special risk for developing PPA-related psychiatric symptoms.

Although PPA has been on the market for over 40 years, it is only recently that the question has arisen as to whether it might produce amphetamine-like mood and behavioural effects; effects which might lead to its abuse. Independent animal experimental studies have indicated that the profile of mood/behavioural effects of PPA is distinct from that of amphetamine (111). Overall data indicate that PPA is a compound of low abuse liability (77, 111-113), which does not produce an amphetamine-like profile of effects.

In massive overdose in association with other drugs, PPA, by causing vasoconstriction may contribute to acute renal failure (114, 115). Three case reports describe renal damage. One patient experienced acute tubular necrosis whilst another, elevated serum myoglobin (116), where the acute renal failure may have been related to rhabdomyolysis.

Caperton (117) reported 3 young women with significant complications of Raynaud's phenomenon which cleared when they discontinued the use of PPA in either cough-cold or diet remedies. He speculated, however, that caffeine may have enhanced possible vasospastic effects.

Phenylpropanolamine has been known to cause blurring of eyesight in some people. A recent report (118) suggests that PPA was the precipitating factor in causing bilateral acute angle closure glaucoma through pupil dilatation in a 37-year old hypermetropic woman.

1.6 INTERACTIONS OF PHENYLPROPANOLAMINE WITH OTHER DRUGS

A major question concerning any active or widely consumed agent is the effect of its use in the presence of disease or other drugs. Many reports of adverse effects following the use of PPA, refer to situations where multiple drugs including PPA were used.

Phenylpropanolamine is subject to the same type of drug interaction as other sympathomimetic amines and, obviously, acceptable doses of other sympathomimetic drugs added to PPA can become unacceptable by summation of effects (119). One of the earliest reported interactions was between monamine oxidase inhibitors (MAOI) and the sympathomimetic amines (81). Several cardiovascular case reports of elevated blood pressure following normal doses of PPA occurred in patients concurrently consuming monamine oxidase inhibitors (MAOI) (120-122).

Concomitant use of an indirect-acting sympathomimetic (IASM) in a patient receiving a MAOI is likely to result in an excessively high blood pressure, severe headache, cardiac arrhythmias, chest pain, circulatory failure, and death has occurred. Administration of MAOI will increase levels of noradrenaline (NA) at its storage site because production of NA is continued without the usual rate of destruction. IASM will release this increased amount of NA from its storage site to act at receptor sites causing a hypertensive crisis (123). A controlled trial was carried out in volunteers to study MAOI-PPA interaction (81). Careful warning instructions accompanying MAOI prescriptions have, however, diminished the likelihood of this, sometimes fatal, interaction.

Beta-blockers may occasionally be responsible for severe hypertension in patients taking large doses of PPA (124). Phenylpropanolamine provoked hypertension in a patient on oxprenolol (125). It was proposed that these interactions result from unopposed adrenergic stimulation in the presence of beta-blockade (126). The mechanism was, however, not supported in a recent study of infused phenylephrine in patients on beta-blockers for hypertension (127).

The antihypertensive action of guanethidine may be blocked by PPA (128), and sensitization to sympathomimetic drugs may be induced following the use of bethanidine whose action is analogous to guanethidine in hypertensive crisis (129, 130). It was proposed that PPA and the antihypertensive agents, including debrisoquine, compete for neural uptake.

A patient on methyldopa and oxprenolol had a hypertensive episode after taking two tablets containing PPA (125). It is believed that beta-blockers permitted the vasoconstrictive effects of PPA to occur, while vasoductor effects were blocked leading to marked hypertension.

One case was reported in which the combination of PPA and cheese, containing tyramine was considered to be the cause of a hypertensive episode, which could have been due to tyramine having an additive effect on the action of sympathomimetic amines (131). In addition, red wines and pickled foodstuffs rich in tyramine might act in conjunction with PPA to cause an adverse cardiovascular event.

Chouinard *et al.* (92) proposed that an unexplained death occurring in a female on thioridazine was due to a ventricular arrhythmia caused by the reaction of a phenothiazine and PPA. However, this association may have been coincidental, since the patient had shown T-wave abnormalities while on chlorpromazine.

More subtle drug interactions may also occur, such as those alleged with indomethacin or other cyclooxygenase inhibitors such as aspirin, that influence the prostaglandin system (132) by reducing the prostaglandin-controlled negative feedback of catecholamine release at sympathetic nerve endings and reducing the amount of vasodilator prostaglandins available.

In one report, two women currently taking oral contraceptives (OC) experienced hypertensive episodes following PPA ingestion (97), and a second report also attributed hypertension to OC/PPA interaction (133). Factors that can cause hypertension from OC use can also increase sensitivity to the hypertensive effects of PPA.

Rumack *et al.* (130) report CNS stimulation and elevated blood pressure in a patient who consumed Ornade (PPA and anticholinergics) and imipramine in PPA's presence. The authors felt that this case represented cumulative overdosage of anticholinergic drugs.

Individuals who are sensitive to or who consume large quantities of coffee may appear to be at greater risk of adverse psychiatric reactions upon administration of PPA, since both caffeine and PPA are sympathomimetics with different, but synergistic mechanisms of action (134). These combinations may also have greater potential to include cardiac arrhythmias and cardiac pathophysiology (98) as well as cardiovascular accident (96). Recently, Schlemmer *et al.* (134) noted that the lethal doses of PPA in rats is decreased when combined with caffeine due to potential of adverse effects and could have contributed to stroke morbidity and mortality in this experiment.

Phenothiazines may antagonize the effect of phenylalkylamines. There is evidence that the administration of general anaesthetics may sensitize the myocardium to the potential for PPA-induced cardiac arrhythmias (128).

1.7 CONTRAINDICATIONS OF PHENYLPROPANOLAMINE

Phenylpropanolamine is contraindicated in pregnancy as it could affect the foetus in pregnant mothers indirectly through effects on uterine blood flow or uterine contractility during labour (135). Drugs studied by Heinone *et al.* (136) with alpha-adrenergic agonist activity were associated with increased risk of malformations.

Since women may be most susceptible to major affective disorders during the postpartum period, expectant and postpartum mothers should be advised against taking any sympathomimetic drugs, including PPA (137). Two women whose cases were reviewed took diet aids during pregnancy and experienced psychotic episodes (138, 139). It has however been found that platelet MAO activity is reduced during pregnancy (140).

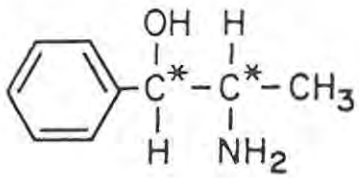
Phenylpropanolamine is contraindicated in patients that have a familial history of psychosis. Susceptible individuals may develop psychotic reactions following the use of OTC diet-aids containing PPA (138). Conditions that impair the balance and control of the sympathetic nervous system can increase susceptibility to the hypertensive effects of PPA. Autonomic dysfunction has been reported to be one such condition (141). This is an idiosyncratic response to PPA. Goldin *et al.* (142) studied the variation in platelet MAO in 309 individuals and found that genetically determined MAO activity, in people with naturally low levels of MAO, can also modify sensitivity to the blood pressure raising effects of PPA.

Phenylpropanolamine should not be used in hyperthyroid, depressed or diabetic patients (143) and is contraindicated in hypertensive and cardiac patients (144).

1.8 ISOMERS OF PHENYLPROPANOLAMINE

Phenylpropanolamine (PPA), chemically known as d, l-norephedrine, is often confused with its various isomers. Confusion about and a nonchalant approach to the distinctions between these products emerged early in their history. Part of the confusion in many reports is the difficulty encountered because of the presumptive, non-probative nature of urine drug screening tests using immunoassays, which depend on a relatively nonspecific antibody. Most of the phenylisopropyl-amines will cross react and a "positive" may reflect the presence of a norephedrine, amphetamine or ephedrine isomer; or even other similar compounds such as phentermine or diethylpropion (145).

FIGURE 1.9 Chiral (asymmetrical) carbons.



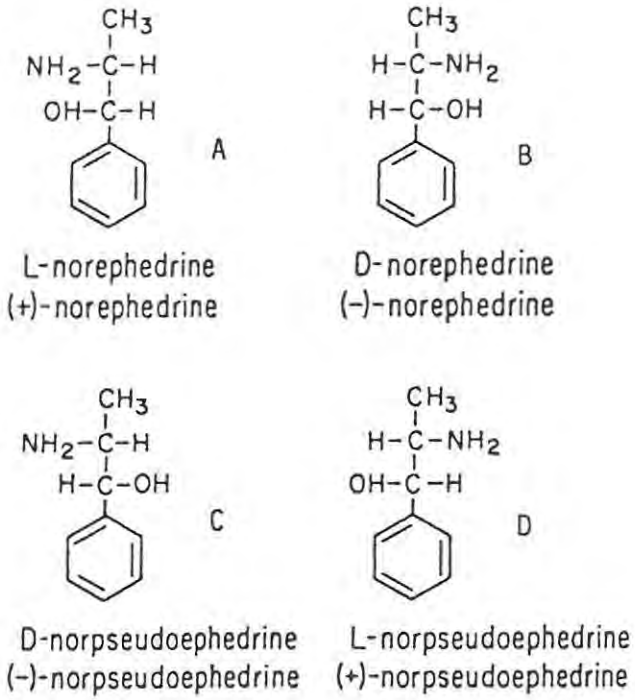
* = asymmetrical carbons

Simplified line drawings (Fig. 1.9) reveal that both the alpha- and beta-carbons are chiral. Therefore PPA has four spatial arrangements and four distinct stereoisomers. Any of these forms or any mixtures of them in pharmaceutical preparations might be called "phenylpropanolamine".

The racemic product most used clinically is (±) norephedrine and is called phenylpropanolamine. Synthetic reactions generally produce racemic mixtures, equal parts of (+) and (-) isomers. Marketed U.S. versions contain a mixture of products A and B in Fig. 1.10. These distinctions are important, because enantiomorphs and diastereomers have different pharmacodynamic properties and effects, and these formulations are not interchangeable despite having been frequently confused.

A recent review of chiral drug action in the rat makes this very clear (146). Results suggested that ℓ-norephedrine and d, ℓ-norephedrine are capable of directly activating vasopressor alpha adrenoceptors, while d-norephedrine had a non-significant effect. It is clear that differences in cardiovascular activity are observed in these structurally similar compounds. Careful attention must thus be given to the proper identification and reporting of these compounds describing their actions and adverse drug reactions because of their significantly different pharmacological properties (147).

FIGURE 1.10 Configurational characteristics of norephedrine isomers.



Reproduced from Morgan (11).

The analytical and preparative resolution of the enantiomers of norephedrine has received a great deal of attention. Analytical methods using indirect (diastereomers) and direct (chiral stationary phases) approaches have been reported (148-151), and preparative resolution of norephedrine have been reported by several investigators (152, 153). A direct enantiomeric high-performance liquid chromatographic (HPLC) resolution of norephedrine has recently been developed by Wainer *et al.* (154).

CHAPTER 2

ANALYTICAL METHODS

2.1 INTRODUCTION

The technique of high-performance liquid chromatography (HPLC), variously called high speed and high pressure liquid chromatography has made a significant contribution to pharmaceutical, biochemical and clinical analysis and is an ideal complementary technique to gas-liquid chromatography. The earliest example of a liquid chromatographic separation is credited to the Russian botanist Tswett who, in 1903, separated plant pigments by adsorption chromatography (156). In 1941, Martin and Synge described the discovery of liquid-liquid partition chromatography (157) and laid the foundation of high-performance liquid chromatography (HPLC), and introduced the concept of the "height equivalent to a theoretical plate" (HETP), which has been adopted as a measure of chromatographic efficiency. Modern HPLC is characterized by short separation times, good sensitivity, precision, and is usually associated with high efficiency and continuous detection of the eluted components (158), rendering it a powerful technique in all facets of analytical chemistry and offers many advantages in drug analysis. Liquid chromatography signifies procedures for the separation of substances that are based on differences in rates of migration arising from different distribution ratios between a flowing mobile phase (liquid) and a stationary phase (solid).

The early columns were essentially similar to those described by Tswett and were based on adsorption chromatography. The breakthrough in HPLC came with the introduction of packing materials in which a stationary phase was covalently linked to silica supports. This resulted in the introduction of the so-called reverse phase system.

A silica support is treated with an organochlorosilane or similar reagent to make a stationary phase of any desired polarity.

$$-SiOH + Cl-SiR_3 \longrightarrow -SiO-SiR_3$$

The most popular supports are those where one of the groups is octadecyl or octyl. They are suitable for all applications where it is important to have no residual interaction with the silicon atoms which are sterically shielded. Apart from the robustness of the new bonded stationary phases, the new columns were suited to drug analysis in biological fluids. First, the drug is likely to be one of the most lipophilic components of the sample and hence is retained on the column longer than most endogenous compounds. Second, the reversed phase mode means that the mobile phase is aqueous, and this is a more appropriate medium for the analysis of an aqueous sample. The use of a partition system rather than an adsorption system gives sharper peaks with less tailing (159).

2.2 ANALYSIS OF PHENYLPROPANOLAMINE RAW MATERIAL AND DOSAGE FORMS

2.2.1 Ultraviolet Spectrophotometric Analysis

Periodate oxidation of PPA.HCl has been used where the sample to be determined is placed in a separatory funnel with $NaHCO_3$ and $NaIO_4$. The solution is extracted with hexane, filtered and the absorbance determined at 242 nm using hexane as the reference solution. The amount of the oxidation product of PPA.HCl is determined by comparison of the sample absorbance against the absorbance of a PPA.HCl reference standard treated in the same manner (160). Other organic solvents such as ether (40) and chloroform (161) have also been used for the extraction of the derivative. Wallace (162) used alkaline periodate oxidation to form benzaldehyde which was subsequently converted to the semicarbazone derivative, thereby enhancing the sensitivity and specificity of the procedure. The drug has also been determined after alkaline extraction into an organic solvent followed by back-extraction under aqueous acidic conditions (163).

2.2.2 Colorimetric Analysis

Phenylpropanolamine hydrochloride can be reacted with ninhydrin in a citrate buffer at elevated temperatures and determined colorimetrically at 570 nm. This reaction has been applied to the determination of PPA.HCl in a multicomponent mixture by an automated system which, after phase separation, utilizes the stream-splitting technique to divide the chloroform stream into segments (164). An ion-pair extraction technique using an acidic dye, bromothymol blue, has been utilized and the resulting chloroform extract determined at 420 nm (165).

2.2.3 Spectrofluorometric Analysis

Phenylpropanolamine hydrochloride has been determined by measuring its fluorescamine derivative, 4-phenylspiro(furan-2(3H),1'-phthal-an)-3, 3'-dione at 480 nm using an excitation wavelength of 398 nm (166). The reaction favours a pH of 9 for optimal reactivity (167, 168).

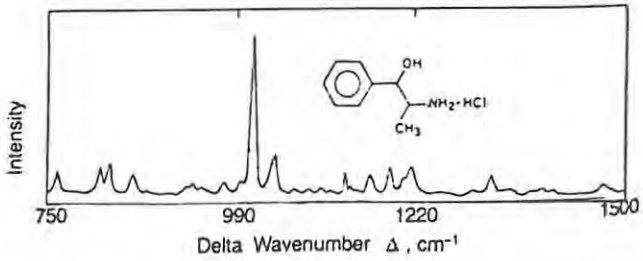
2.2.4 Titrimetric Analysis

After extraction of PPA.HCl from an alkaline aqueous solution with chloroform, shaking with saturated NaCl solution and back-extracting with an excess of H_2SO_4, the excess acid is titrated with a standard NaOH solution using methyl red as indicator (169).

2.2.5 Raman Spectroscopic Analysis

Both solution and solid samples of PPA.HCl in a multicomponent mixture have been determined quantitatively by Raman Spectroscopy (170), without prior separation. A survey scan of PPA.HCl is shown in Fig. 2.1.

FIGURE 2.1 Survey scan of phenylpropanolamine hydrochloride



2.2.6 Chromatographic Analysis

2.2.6.1 Column Chromatography

A weakly basic anion exchange resin, Amberlite IR-45, was found to be suitable for the separation of PPA.HCl from various dosage forms and yielded a 99.6% recovery of the drug which was then determined titrimetrically (171). Being a nitrogenous base, the drug is retained on a sulphonated polystyrene cation exchange resin. Determination is then effected by measuring the ultraviolet absorbance after elution with HCl (172, 173). The drug has also been determined by mixing with NH_4COH, eluting with chloroform from a Celite column and measuring the absorbance at 258.5 nm (174).

Separation of PPA.HCl from mixtures of drugs in various dosage forms has also been described (175). The method involves the retention of PPA on the first of four Celite columns followed by elution, addition of NaOH, extraction of the free base with chloroform, back-extraction using H_2SO_4 and subsequent determination at 257 nm using H_2SO_4 as the reference solution. An on-column periodate oxidation of PPA to benzaldehyde has also been described and the derivative determined at 267 nm (176).

2.2.6.2 Paper Chromatography

A descending paper chromatographic technique using Whatman No. 1 paper has been reported. The solvent system consisted of a 1:1 mixture of butanol (saturated with 1M HCl) and methanol. After spraying with Dragendorff's reagent, the resulting orange-red spots were quantitatively determined by photoelectric densitometry (177).

2.2.6.3 Thin Layer Chromatography

A number of thin layer chromatographic systems have been described for PPA.HCl and these are listed in Table 2.1. Phenylpropanolamine is detected by spraying with reagents to form a coloured or fluorescent spot, or by overspotting the drug with a derivatizing agent. These procedures are necessary as PPA has a poor inherent ultraviolet absorptivity and consequently is difficult to detect directly.

TABLE 2.1 Thin Layer Chromatographic systems for Phenylpropanolamine

Solvent System	Adsorbant	Detection	Rf	Ref
Chloroform layer from a mixture of chloroform/acetic acid/methanol/water (85 : 20 : 8 : 20)	Silica Gel G (F254) (60)	Sprayed with 0.3% p-nitroaliline plus 5% sodium nitrite, heated at 70°C for 15 minutes then sprayed with 20% Na_2CO_3	Not reported	161
Not reported	Silica Gel G (F254) (60)	Sprayed with buffer (pH 9.3), over-sprayed with fluorescamine/acetone solution and resprayed with buffer	0.50	167
Benzene/ethyl acetate (70 : 30)	Gelman ITLC fibre SAF	p-nitrobenzoyl chloride applied and heated (100°C)	0.79	178
Benzene/ethyl acetate (30 : 70)			0.90	
Hexane/ethyl acetate (50 : 50)			0.76	
Hexane/ethyl acetate (30 : 70)			0.90	
Ethyl acetate/methanol/ formic acid (69 : 30 : 1)	HPTLC Silica Gel 60	Dipped into o-phthalaldehyde solution followed by 20% polyethylene glycol in methanol	0.40	179
n-butanol/acetic acid/ water (4 : 1 : 5)	DC-cellulose	Ninhydrin reagent	Not reported	180
Heptane/methanol (60 : 40)	HPTLC Silica Gel 60	Fluorescamine solution	0.45	181
Benzene/methanol (83 : 17)			0.37	
Benzene/acetone/methanol/ dioxane (40 : 40 : 4 : 5)			0.28	
Ethyl acetate/methanol/ water/ammonia (85:10:3:1)	Silica Gel	Sprayed with 0.3% ninhydrin acid, heated at 100 C for 5 minutes, sprayed iodoplatinate reagent then p-nitroaniline reagent, heavily sprayed with 25% alcoholic NaOH solution	Not reported	182
Chloroform/methanol (4:1)	Silica Gel G	Iodine vapour	0.17	183
Chloroform/methanol (4:1)	Silica Gel G (HF254)	Iodine vapour	0.14	

2.2.6.4 Gas Chromatography

Gas chromatography has been extensively used for determining PPA in pharmaceutical preparations and, to a lesser extent, for the determination of the drug in biological fluids. The drug has been chromatographed directly without derivitization and also as the silyl, pentafluorophenyloxazolidine, acetone, butanone, trifluoroacetyltrimethylsilyl, haptafluorobutyryl and the 2, 6-dinitro-4-trifluoromethylbenzenesulphonic acid derivatives. The gas chromatographic conditions are listed in Table 2.2.

TABLE 2.2 Gas Chromatographic Systems for Phenylpropanolamine

Drug Source	Column	Carrier Gas	Column Temp(°C)	R_T	Detector	Injected gas	Ref
Tablets, syrup	2.0m x 4.0mm (i.d.) glass 0.1% silicone oil (DC-710) on 60-80 mesh dimethyldichlorosilane treated glass beads	He	200	2.80	FID	Silyl derivative	184
Syrup	2.4m x 3.2mm (o.d.) Pyrex glass 2% SE-30 on Chromosorb W (HP)	He	180	1.80	FID	Phenylpropanolamine	185
Tablets	1.8m x 4mm glass 3% OV-17 on Gas Chrom Q	He	230	0.85	FID	Phenylpropanolamine	186
Tablets, capsules, liquids	1.8m x 6.4mm (i.d.) glass 1% HI-EFF-8BP and 10% SE52 on Gas Crom Q	N_2	220	2.40	FID	Phenylpropanolamine hydrochloride	187
	4% HI-EFF-8BP on Gas Chrom Q		220	1.25			
Raw material	1.4m x 4mm (i.d.) glass 3% Poly A 103 on Gas Chrom Q	He	Programmed 70-250	28.00	FID	Trifluoroacetyltrimethylsilyl derivative	188
Raw material	1.1m x 2.5mm glass 18.8% Apiezon N on Diatoport S	N_2	180	2.55	FID	Phenylpropanolamine	189
			138	2.77			
			101	3.18			
Raw material	1.8-2.4m x 3mm (i.d.) glass 1.15% SE-30 on Gas Chrom P	Ar	104	9.10	EC	Phenylpropanolamine	190
				13.40		Acetone derivative	
				123.50		Butanone derivative	

Raw material	1.8m x 2mm (i.d.) glass 3% OV-1 on Supelcoport 0.9m x 2mm (i.d.) glass 3% SP-2250 on Supelcoport	N_2	250 220 230	1.38 1.38 0.98	EC	2,6-dinitro-4-trifluoro-methylbenzene sulphonic acid derivative	191
Raw material	1.2m x 4mm (i.d.) glass 2% SE-30 and 2% Carbowax 20M on Anachrom ABS	N_2	185	1.60	FID	Phenylpropanolamine hydrochloride	192
Raw material	1.8m x 2mm (i.d.) glass 3% OV-17 on Anachrom ABS	He	Programmed 100-250	8.36	Nitrogen	Phenylpropanolamine	193
Biological material	1m x 6mm (o.d.) glass 7.5% Carbowax 20M on Chromosorb W 2.0% Carbowax 20M on Chromosorb G	N_2	165 120	4.10 1.10	FID	Phenylpropanolamine	183
Capsules	1.8m x 2mm (i.d.) glass 8% OV-101 on Chromosorb W-HP	N_2	290	7.00	FID	Acetyl derivative	194
Capsules, suspensions	183cm x 2mm (i.d.) glass 3% OV-225 on Gas Chrom Q	He	190	Not reported	FID	Phenylpropanolamine	195

2.2.6.5 High-Performance Liquid Chromatography

Methods and the associated conditions for the high performance liquid chromatographic determination of PPA alone and in pharmaceutical formulations are listed in Table 2.3. Relatively little has been published on the HPLC analysis of PPA in biological fluids (see Section 3.1).

TABLE 2.3 High Performance Liquid Chromatographic Systems for Phenylpropanolamine.

Drug Source	Column	Mobile Phase	Flow rate (ml/min)	R_T (mins)	Detection	Ref
Tablets, syrup	0.5m x 2.1mm (i.d.) Du Pont Zipax SCX	0.02M $(NH_4)_2HPO_4$ in 36% dioxane/water	1.0	2.2	Stated only as u.v.	196
Syrup	0.3m x 4mm (i.d.) Waters μ Bondapak C_{18}	0.05M KH_2PO_4 in water containing 13% (v/v) methanol	2.0	3.0	254nm	197
Tablets, capsules, liquid	0.3m x 4mm (i.d.) Waters μBondapak phenyl	Water/methanol/acetic acid (55:44:1) containing 0.005M sodium heptane sulphonate	2.0	not stated	254nm	198
Tablets liquid	0.3m x 4mm (i.d.) Waters μ Bondapak CN	13% acetonitrile in water containing 1.8% acetic acid	Gradient elution	7.5	254nm	199
		13% acetonitrile in water containing 1.8% acetic acid and 0.005M sodium heptane sulphonate		8.8		
Tablets	0.25mx4.6mm (i.d.) Whatman Partisil-10-ods	2.85 x 10-3M ethylenediamine buffer (pH 7.44)/acetonitrile (1:1)	3.8	3.3	216.5nm	200
Syrup	0.3m x 3.9mm (i.d.) Waters μBondapak phenyl	Methanol/water (60:40) with 0.004M sodium heptane sulphonate and 1% acetic acid	1.0	7.4	254nm	201
	Water μBondapak C_{18}		1.0	11.7		
Syrup	0.15m x 4.6 mm (i.d.) Spherisorb S5W	0.1M KH_2PO_4 in 10% aqueous ethanol	1.0	5.4	198nm	202
Spray	0.3m x 4mm (i.d.) Waters μBondapak C_{18}	Methanol/water (50:50) with PIC reagent B-7		3.1	254nm	203
Syrup	0.25m x 4.6mm (i.d.) Zorbax TMS column	hexanesulphonic acid/acetic acid/water/methanol (0.1:1:68.9:30)	2.5	2.4	254nm	204
Liquid	0.3m x 4mm (i.d.) Waters μ Bondapak C_{18}	methanol/water/tetrahydrofuran 80% phosphoric acid (67:29:4: 0.1) with 5.8g dioctylsulfo-succinate adjusted to pH 3.8	1.3	7.1	254nm	205

Tablets, capsules, liquid	0.25m x 4mm (i.d.) Waters μBondapak C_{18} 0.25m x 4.6mm (i.d.) Whatman Partisil-10 C_8	Methanol/water (35:62.5) with 2.5ml PIC reagent B-5 Methanol/water (30:67.5) with 2.5ml PIC reagent B-5	2.0 2.0	5.1 5.8	254nm	206
Tablet, syrup	8mm x 10cm Radial-PAK Porasil Silica	Methanol/water 0.01M $(NH_4)_2HPO_4$ (75:25)	4.0	2.5	254nm	207
Tablets, liquids	30cm x 4mm (i.d.) Waters μBondapak phenyl	Aqueous KH_2PO_4 buffer, 0.02M (pH 2.6) with 85% aqueous solution phosphoric acid (0.4 mL/L)	2.5	4.0	256nm	208
Capsules, Tablets	25cm x 4.6 mm (i.d.) Whatman Partisil-10 C_8	Methanol/Acetonitrile/pentanesulfonic acid sod. 0.005M in glacial acetic acid (1.7%) (5:17:75.5:2.5)	2.0	4.9	254nm	209
Tablets, capsules	25cm x 4.6mm (i.d.) Whatman Partisil-PXS ODS-2 10 m	Acetonitrile/water (30:70)	1.7	8.3	254nm	210

* Injected as the sodium metaperiodate derivative in alkaline medium

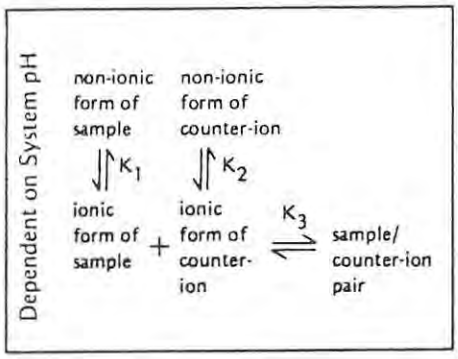
2.3 DEVELOPMENT OF AN HPLC METHOD FOR THE DETERMINATION OF PHENYLPROPANOLAMINE

INTRODUCTION

An adaptation of a published high-performance liquid chromatography (HPLC) assay for phenylpropanolamine was used to examine PPA pharmacokinetics. Because this particular analytical method for serum and urine PPA determinations has not previously been reported, the method validation data are also presented (See Chapter 3).

A relatively new liquid chromatography (LC) variation, paired-ion chromatography (PIC), was developed to solve the analytical problems traditionally associated with samples containing ionic samples thereby considerably increasing retention for these compounds. The resulting species can be easily chromatographed by a reverse-phase LC system.

The following equilibria explain this phenomenon (203).



For basic samples, alkyl sulfonates (pKa<1) are used. The pKa of an alkyl sulfonic acid counter-ion should be less than pH 3 for ion pair formation with bases so that the counter-ion will be in an ionic form at any pH required to drive the sample to its ionic form and hence K_2 will be negligible. Minimum sample preparation and extended column lifetimes characterize permanently bonded reverse-phase LC systems for quantitative analysis. Variables include solvent polarity, pH, type and concentration of counter-ion and, to an extent, the stationary phase. The variables give additional possibilities for optimizing selectivity. The method is a modification of the HPLC method reported by Dowse *et al.* (211).

2.3.1 Experimental

2.3.1.1 Reagents

All chemicals and reagents used were of at least analytical grade and used without further purification. The sodium salt of 1-heptanesulfonic acid[1] was used as received. The acetonitrile[2] was distilled-in-glass grade. The HPLC water used in the mobile phase was purified through a Milli-Q purification system (Waters Associates). Phenylpropanolamine hydrochloride[3], ephedrine hydrochloride[4], hydrochloric acid[5], sodium salts of 1-octanesulfonic acid and 1-dodecanesulfonic acid[6] were obtained commercially.

The mobile phases were filtered through 0,6 µm cellulose acetate filters[6] and deaerated under vacuum.

1. Sigma Chemical Co., St Louis, USA.
2. Burdick and Jackson Lab. Inc., Muskegon, Mich., USA.
3. Aldrich Chemical Co., Milwaukee, Wis., USA.
4. Holpro Analytics (Pty) Ltd., Johannesburg, South Africa.
5. BDH Chemicals Ltd., Poole, England.
6. Type BD, Millipore Corp., Bedford, Mass., USA.

Solutions of drug samples were prepared in A-grade volumetric flasks by the addition of HPLC grade water.

2.3.1.2 The High-Performance Liquid Chromatographic Systems

SYSTEM A

The chromatographic system consisted of an M45 solvent delivery system (Waters Associates), a model U6KB injector (Waters Associates), a model 1238 LKB fixed wavelength Uvicord S UV detector and a model 560 strip-chart recorder (Perkin-Elmer). Separation was achieved on a 25 cm x 3.9 mm i.d. Techsil C_{18} (10 µm) reverse phase column (HPLC Technology). Temperature was ambient.

SYSTEM B

This consisted of M45 solvent delivery system (Waters Associates) and a model U6K injector (Waters Associates). The effluent from a 25 cm x 3.9 mm i.d. Techsil C_{18} (10 µm) reverse phase column (HPLC Technology) was monitored with a 1040A Diode-Array detector (Hewlett Packard) to which was attached a model R100A strip-chart recorder (Perkin-Elmer). The temperature of the column was controlled by an LC-22 temperature controller and column heater (Bioanalytical Systems Inc.).

SYSTEM C

This system incorporated an M45 solvent delivery system (Waters Associates), a WISP model 710B automatic sample injector (Waters Associates), a variable wavelength LC3-UV detector (Pye Unicam) and a model 561 strip-chart recorder (Perkin Elmer). A model LC-22 temperature controller (Bioanalytical Systems Inc.) was used to maintain the Techsil C_{18} (10 µm) reverse phase column (HPLC Technology) at the desired temperature. A Techsil 25 cm x 3.9 mm i.d. C_{18} reverse phase (5 µm) column (HPLC Technology) was used in the later stages of development.

SYSTEM D

The final HPLC system used throughout analysis of biological samples consisted of

1) Model SP8780XR microprocessor-controlled modular automatic liquid sampling unit (Spectra-Physics).
2) Model 114M solvent delivery system (Beckman Instruments Inc.).
3) Model SP4290 Integrator (Spectra-Physics).
4) Model SP8480XR UV-Visible scanning detector (Spectra-Physics).

Separation was achieved on a 25 cm x 3.9 mm i.d. Techsil C_{18} (5 µm) reverse phase column (HPLC Technology) and a model LC-22 temperature controller (Bioanalytical Systems Inc.) maintained the column at the desired temperature.

2.3.1.3 Ultraviolet (UV) Detectors

The liquid chromatography detector is the key to obtaining qualitative and quantitative information about the analytes separated. The UV absorption detector which is relatively insensitive to temperature and flow variations is the most widely used detector in HPLC owing to its high stability, sensitivity and linear range.

1) The UV-Visible Scanning detector SP8480XR (Spectra-Physics)

This detector is characterized by its high sensitivity, low noise, and reliability. It is ideally suited for a variety of applications and can be used over a broad range of conditions, all a result of the combination of a wide dynamic range and high-speed detector electronics. The detector is fitted with a 12 µL volume flow cell, and comes with software for the SP4290 integrator. This detector was operated at 210 nm at the maximum sensitivity setting of 0.001 absorbance units full scale (AUFS).

2) The 1040A Diode-Array Detector (Hewlett Packard)

This detector which has a standard 3.5 µL flow cell was controlled and assisted by the following components: An HP 85 personal computer, an HP 2191 dual disc drive and an HP 7470 X-Y plotter. It has the facilities of a diode-array detector with the additional advantage of a data storage facility which allows post-run manipulation of chromatograms as well as the ability to monitor and permanently store the results of chromatograms at eight different wavelengths. The detector was usually set to simultaneously monitor four different wavelengths; 205, 210, 215 and 220 nm with the sensitivity adjusted to give the best signal/noise ratio. It may be used for the scanning of peaks and generation of 3-dimensional plots of absorption vs time vs wavelength.

3) The LC3 variable wavelength ultraviolet detector(Pye Unicam)

The Pye Unicam LC3 UV detector is a reference compensated variable wavelength single beam ultraviolet absorbance monitor, designed for monitoring the eluent from the HPLC column. The instrument reads out in absorbance over the wavelength range 190 to 380 nm. The variable wavelength facility is useful for optimizing the instrument sensitivity. Additional facilities included are recorder zero and adjustment for maximum energy throughput. The detector has an 8 µL volume flow cell. After injecting aqueous samples of PPA.HCl at wavelengths ranging from 200 nm to 250 nm, it was found that 210 nm yielded the most stable baseline with a satisfactory response.

2.3.1.4 Columns and Mobile Phases

(i) C_{18} Reverse Phase Column (10 µm)

This, the first column used, was a 30 cm x 4 mm i.d. steel column packed with Techsil 10 µm octadecylsilane (C_{18}) material (HPLC Technology) by the method described in section 2.3.8. Preliminary studies were performed on this column. These included the effect of ion-pair reagent strength and mobile phase composition on the retention of PPA. The choice of the optimum mobile phase and operating conditions for this column are summarized as systems 1 and 2 (Table 2.4).

(ii) C_{18} Reverse Phase Column (5 µm)

A 30 cm x 4 mm i.d. column was packed with Techsil 5 µm C_{18} material. The optimum operating conditions for this column are summarized in systems 3 and 4 (Table 2.4). When the formation of a top-end void became evident it was repaired by application of a paste of Techsil 5 µm C_{18} in methanol.

(iii) C_{18} Reverse Phase High Speed Column (3 µm)

This commercially available high speed column was only 7.5 cm long with an internal diameter of 4.6 mm. It was packed with 3 µm C_{18} material (HPLC Technology). Numerous mobile phases, differing in organic/aqueous phase ratios and ion-pair reagent strength operated at flow rates between 1.0 and 2.5 mL/min were tested to obtain a suitable separation between PPA and the internal standard. The best combination of these variables is reported in system 5 (Table 2.4).

2.3.1.5 Guard Columns

The use of a guard column packed with 20-40 micron LC-18 pellicular packing (Supelco Inc., Pennsylvania) was initiated at various stages during development as biological samples were being injected directly onto the column. The guard column serves to trap particulate matter and high molecular weight constituents such as protein, thereby prolonging the life of the analytical column. The 5 µm analytical

column was protected by a 10 µm C_{18} cartridge guard column (HPLC Technology Ltd., Part no. 10C18) housed within a cartridge holder (HPLC Technology Ltd., Part no. GTL-EL3101) so that the non-eluting materials in the samples injected were retained. The guard column also absorbs the pump's pressure surges which damage the packing, and serves as a buffer, protecting the HPLC column against the negative influences of the sample and the LC system. The guard column demonstrated an absence of significant contributions to dead volume or extra column effects. When the guard column cartridge lost efficiency or caused double peaks it was disposed of and replaced by a new cartridge obtained commercially.

TABLE 2.4. High Performance Liquid Chromatographic Systems used.

SYSTEM	HPLC SYSTEM	COLUMN	MOBILE PHASE	FLOW RATE (mL/min)	TEMPERATURE (°C)	PRESSURE (psi)
1	B	C_{18} REVERSE PHASE 10 µm (TECHSIL)	0.005M HSS solution/ acetonitrile (75/25) with 0.2% 1M HCl solution	1.2	Ambient	1700
2	C	C_{18} REVERSE PHASE 10 µm (TECHSIL)	0.005M HSS solution/ acetonitrile (74/26) with 0.2% 1M HCl solution	1.2	Ambient	1700
3	C	C_{18} REVERSE PHASE 5 µm (TECHSIL)	0.005M HSS solution/ acetonitrile (75/25) with 0.2% 1M HCl solution	1.2	30	2600
4	D	C_{18} REVERSE PHASE 5 µm (TECHSIL)	0.005M HSS solution/ acetonitrile (77/23) with 0.15% 1M HCl solution	1.2	30	2540
5	E	C_{18} REVERSE PHASE HIGH SPEED 3 µm	0.005M HSS solution/ acetonitrile (74/26) with 0.25% 1M HCl solution	1.2	Ambient	1900

2.3.1.6 Mobile Phase Preparation

The mobile phase was prepared by mixing acetonitrile (270 mL) with a 0.005 M solution 1-heptanesulfonate (HSS) in water (900 mL) and adding 1 M HCl (1.8 mL). The 0.005 M sodium 1-heptanesulfonate solution was freshly prepared by dissolving 1.011 g of the salt in 1.0 litre HPLC grade water. The solvent mixture (pH 2.68) was filtered under vacuum to expel dissolved gases. Adequate time (about 1 hour) was allowed for the system to fully equilibriate before starting analysis. The mobile phase was recycled, resulting in a more stable baseline and minimal baseline drift. At the completion of a one-day run, the column was flushed to remove any residues or halides that are detrimental to the stainless steel components of the HPLC apparatus. Flushing was accomplished by pumping about 100 mL methanol/water (50/50) through the system.

2.3.1.7 Column Packing Procedure

Columns were packed as follows using a pneumatic column packing pump. Packing material from a used, 25 cm x 3.9 mm i.d. steel casing (μBondapak, Waters Associates) was removed and the column thoroughly rinsed with methanol. The column end-frits and sieves were sonicated for 20 min in methanol. Three grams of packing material (Techsil C_{18}, 5 or 10 μm) was slurried in 50.0 mL HPLC-grade carbon tetrachloride and sonicated until dispersed. After loading the carbon tetrachloride slurry into the column packing reservoir, 150.0 mL of degassed and filtered HPLC grade methanol was pumped through the column at a pressure of 5 000 psi. After this period the column was inverted without interruption of flow and a further 150.0 mL methanol was pumped through the column before removal from the column packer. The column was then removed from the packing system and washed with 50.0 mL acetonitrile/water (75/25) and tested using this mobile phase and a standard laboratory mixture prepared as follows:

Ten milligrams of both benzamide and benzephenone, plus 0.5 mL benzene were made up to 20.0 mL with acetonitrile. One millilitre of this solution was diluted to 50.0 mL with acetonitrile/water (75/25).

Two millilitres of this solution were injected onto the column at a flow rate of 1.0 mL/min, with U.V. detection at 254 nm, a sensitivity setting of 0.04 AUFS and a chart speed of 1 cm/min.

2.4 RESULTS AND DISCUSSION

2.4.1 Detector Wavelength

Phenylpropanolamine absorbs in the low UV wavelength range but its inherent molar absorptivity is poor (34). The need to detect low concentrations of PPA in biological fluids (10.0-500 ng/mL) necessitated the choice of a wavelength where the absorbance was relatively high. From Fig. 1.6 it can be seen that PPA only significantly absorbs U.V. radiation below 220 nm with a wavelength of 200 nm giving the highest absorptivity. A wavelength of 220 nm has been shown by Dowse *et al.* (211) to provide sufficient sensitivity when monitoring PPA concentrations of 25 ng/mL and more. The need for maximum sensitivity led to the choice of 210 nm as the wavelength of detection, as wavelengths below 210 nm yielded unacceptable baseline noise and drift, while those above did not provide sufficient sensitivity. Figure 2.2 depicts the decrease in detector sensitivity resulting from an increase in detector wavelength from 200 to 250 nm after injecting aqueous samples (20 µL) of PPA.HCl (10 µg/mL).

2.4.2 Detector Choice

The Spectra-Physics detector was found to have the most favourable signal/noise ratio at 210 nm when set at its maximum sensitivity of 0.001 AUFS, and was used for the majority of analyses throughout this study. As a result it was used as a standard for the comparison of various detectors as previously described. Chromatograms of PPA using the Hewlett-Packard 1040A, Pye Unicam LC3 and Spectra-Physics SP8480XR UV detectors are shown in Fig. 2.3.

FIGURE 2.2 Chromatogram of PPA (1) monitored simultaneously at seven different wavelengths.

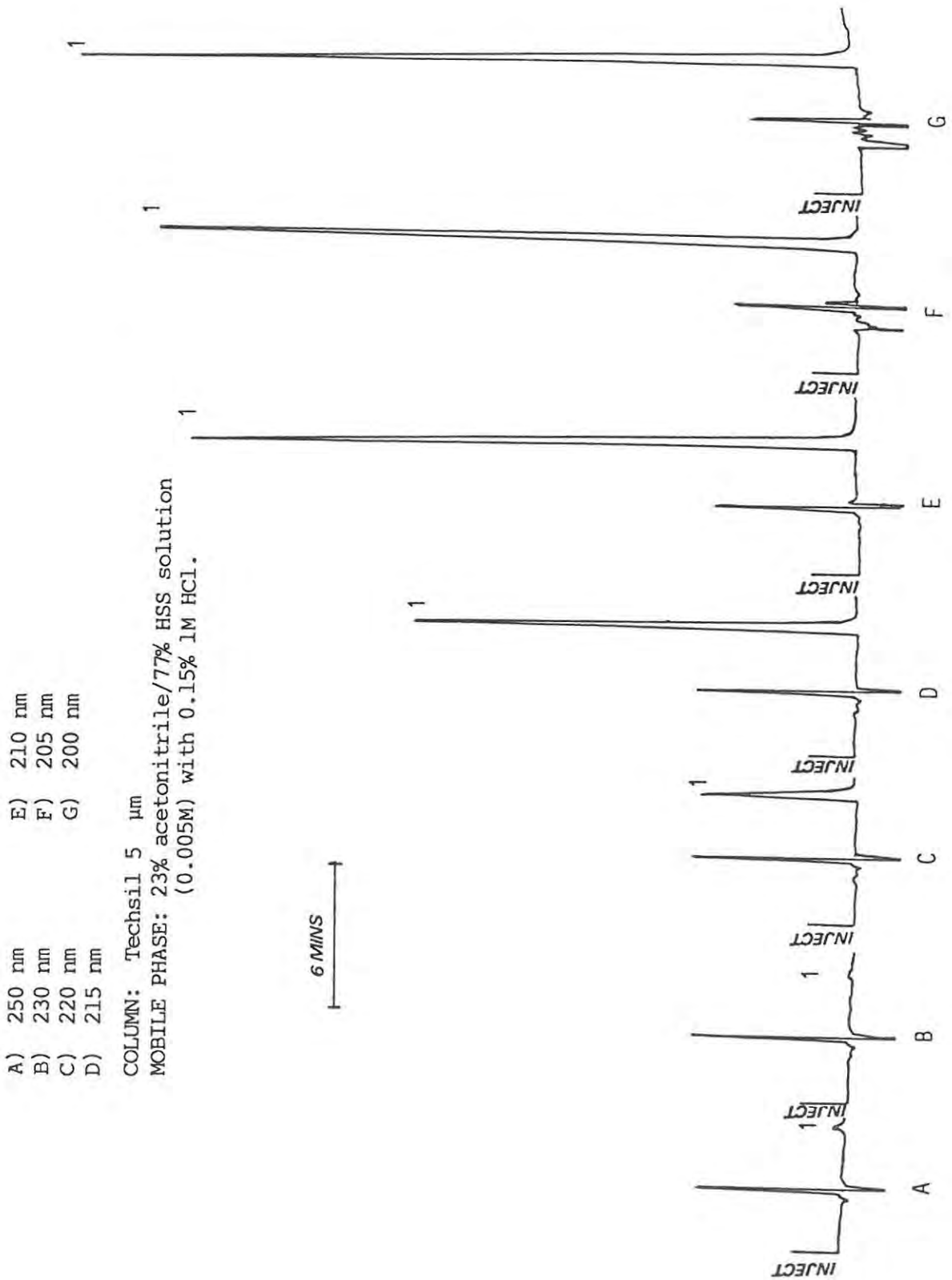
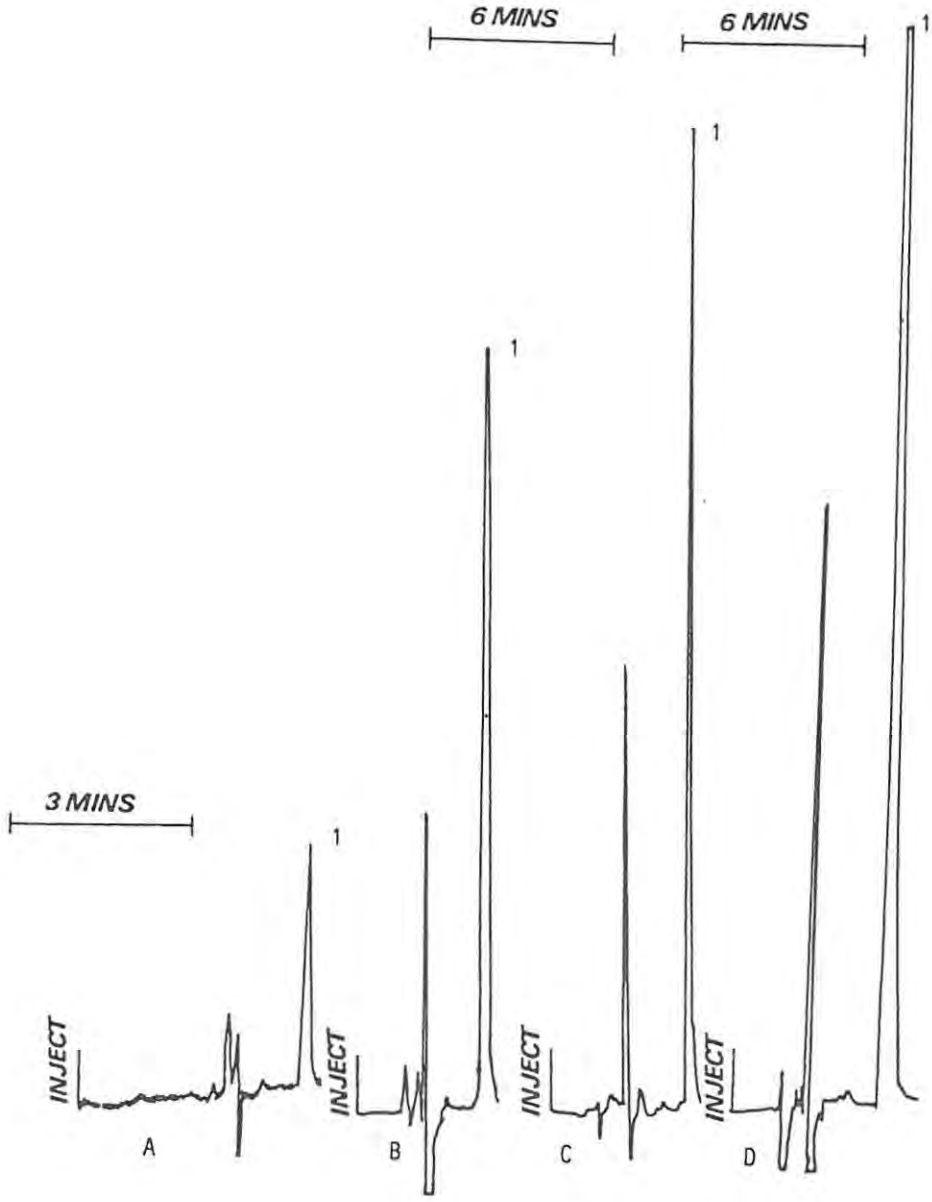


FIGURE 2.3 Chromatograms of PPA (1) using three different detectors

A) Hewlett-Packard 1040A
B), C) Pye Unicam LC3
D) Spectra-Physics SP8480XR

COLUMNS: A), B) 10 µm Techsil
C), D) 5 µm Techsil

MOBILE PHASE: 23 % acetonitrile/77 % HSS solution (0.005 M) with 0.15 % 1 M HCl.
pH = 2.65



2.4.3 Internal Standard

The compound chosen as the internal standard should preferably have physico-chemical properties as close as possible to those of the analyte. Homologous or analogous compounds and isomers are preferred to other substances (158). The detector response and extraction properties of the internal standard were evaluated in parallel to that of the analyte. Ephedrine hydrochloride (EPH.HCl) was chosen as the internal standard. It was readily detectable at 210 nm, and eluted after PPA. Endogenous compounds which eluted just before PPA did not interfere with the EPH peak. Peak shape was sharp and symmetrical and resolution between PPA and EPH peaks was satisfactory on the final chromatographic system used.

2.4.4 Mobile Phase Selection

The necessity of having an ion-pairing agent in the mobile phase for the elution of PPA from a C_{18} column was established in preliminary studies (211). Three ion-pairing agents, namely the sodium salts of 1-heptanesulfonate, 1-octanesulfonate or 1-dodecanesulfonate were included in the mobile phase. The compounds pair with PPA to form a neutral, non-polar complex. The elution order is often predictable based on the degree of hydrophobicity of the solute. The more hydrophobic, the greater is the chromatographic retention of the ion-pair.

Baseline resolution was obtained with all three agents, but broad peaks with long retention times resulted using 1-octanesulfonate sodium (0.005 M), and deterioration in peak shape and height resulted from the mobile phase containing 1-dodecanesulfonate sodium (0.001 M) (Fig. 2.4). 1-heptanesulfonate sodium (HSS) in the mobile phase (0.005 M) yielded good baseline resolution.

After varying concentrations of the counter-ion, 1-heptanesulfonate sodium (HSS), it was found that a molarity of 0.005 M yielded the most satisfactory retention time and resolution (Fig. 2.4).

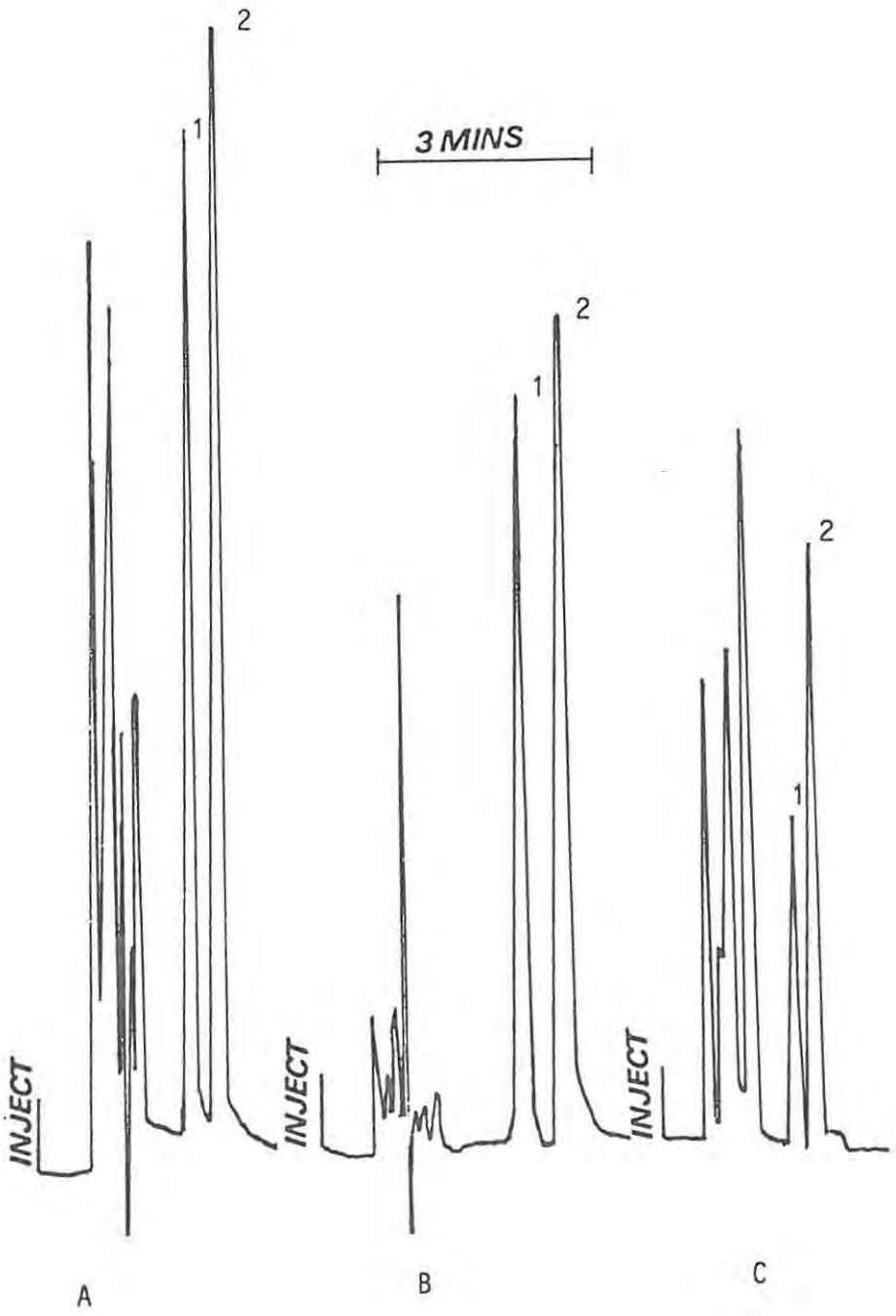
FIGURE 2.4 The effect of ion-pairing agents in the mobile phase on the retention of PPA (1) and EPH (2).

COLUMN: 10 µm Techsil

MOBILE PHASES: A) 26 % acetonitrile/74 % Heptanesulfonate sodium solution (0.005 M) with 0.2 % 1 M HCl

B) 26 % acetonitrile/74 % Octanesulfonate sodium solution (0.005 M) with 0.2 % 1 M HCl

C) 26 % acetonitrile/74 % Dodecanesulfonate sodium solution (0.0025 M) with 0.2 % 1 M HCl



Since it was necessary to decrease the pH of the mobile phase to reduce tailing of the compounds of interest, hydrochloric acid was included as this did not interfere with detection at 210 nm. However, a pH range of 2-7 is recommended for monomeric C_{18} columns, and thus not more than 0.2% 1M HCl was used in the mobile phases. In addition, halides are detrimental to the stainless steel components of the HPLC apparatus. Care was taken to flush the system each night (see section 2.3.1.7). Baseline resolution was obtained using 25% methanol/75% HSS solution (0.004 M), but broad peaks resulted, with long retention times (Fig. 2.5).

Acetonitrile (HPLC grade) was used in place of the methanol component. An acidified mobile phase consisting of 22.5% acetonitrile/77.5% HSS solution (0.005 M) afforded resolution at ambient temperatures, although a small amount of baseline drift occurred. Increasing the temperature had little effect on resolution, but the maintenance of constant temperature (30°C) decreased this baseline drift. A column heater set at 30°C was used for all further determinations. After varying proportions of acetonitrile/aqueous HSS solution (0.005 M) in acidified mobile phases, a final mobile phase consisting of 23% acetonitrile/77% HSS solution (0.005 M) with 0.15% 1.0 M HCl was found to be satisfactory at 30°C and was used for the determination of all serum and urine extracts (Fig 2.6).

2.4.5 Column Choice

The 25 cm column packed with 10 µm C_{18} material (Techsil) was found to be adequate for the initial studies of the effects of mobile phase composition on resolution. However, higher column efficiency and sample sensitivity was required, which led to the choice of a column of the same length packed with 5 µm C_{18} material (Techsil). The principal reason for using the 5 µm packed column was to obtain greater separation efficiency, and was subsequently used for the determination of all serum and urine extracts. The 5 µm packed column could be operated at relatively modest back pressures (2.54 kpsi) and column lifetime was approximately 10 months. Precautions were taken to prolong column life. To minimize instrument error, a system

FIGURE 2.5 Chromatogram of PPA (1) and EPH (2)

COLUMN: 10 µm Techsil

MOBILE PHASE: 25 % methanol/75 % HSS solution (0.004 M) with 0.2 % 1 M HCl. pH = 2.71.

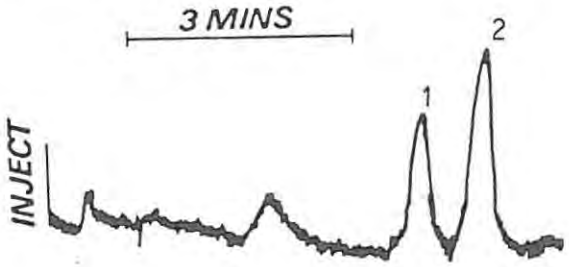


FIGURE 2.6 Chromatogram of PPA (1) and EPH (2)

COLUMN: 5 µm Techsil

MOBILE PHASE: 23 % methanol/77 % HSS solution (0.005 M) with 0.15 % 1 M HCl.
pH = 2.65

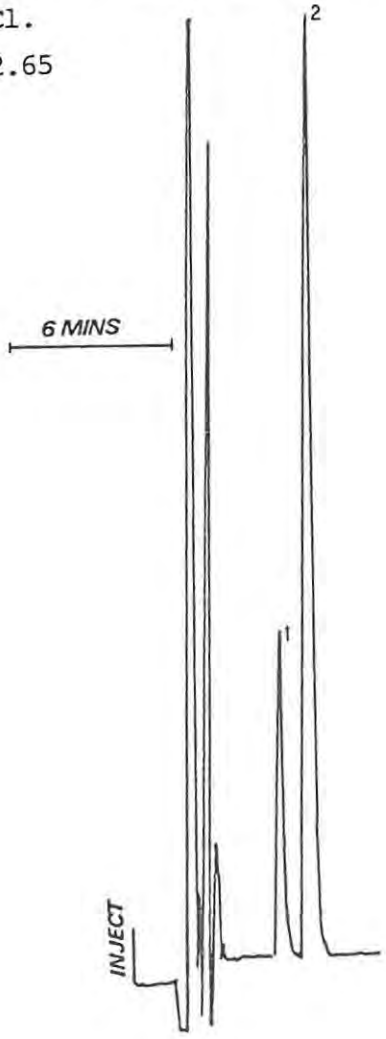
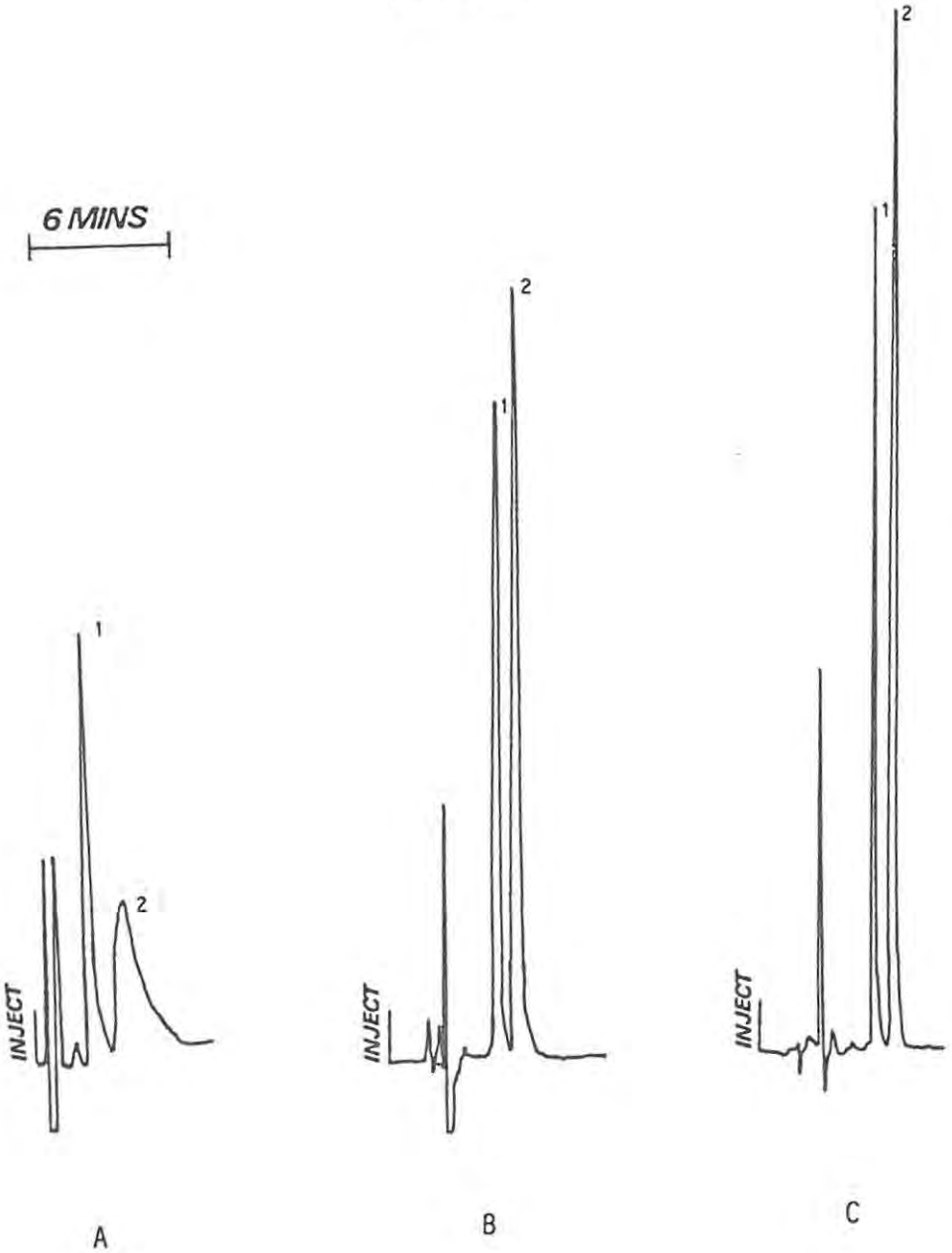


FIGURE 2.7 Chromatograms of PPA (1) and EPH (2)

COLUMNS
A) 3 µm Techsil
B) 10 µm Techsil
C) 5 µm Techsil

MOBILE PHASE
A), B) 25 % acetonitrile/75 % HSS solution (0.005 M) with 0.2% 1 M HCl solution, pH=2.51
C) 74 % acetonitrile/ 26 % HSS solution (0.005 M) with 0.2% 1 M HCl solution, pH = 2.65



stability test with repetitive injections of a test mix were part of the HPLC method protocol. The C_{18} high speed column (3 µm) proved to be less efficient than both the 10 µm and 5 µm columns using a similar mobile phase composition, and resulted in peak broadening of the EPH peak (Fig. 2.7). An assay procedure for the analysis of PPA and EPH could be developed using the high speed 3 µm column used in this study with small changes in mobile phase composition.

2.4.6 Effect of Flow Rate

The effect of flow rate on resolution was investigated early in the development programme. Although improved resolution may be achieved by reduction of flow rate (212), it was found that flow rate had little effect on peak resolution.

An increase in flow rate from 0.5 to 1.2 mL/min in 0.1 mL increments provided separation and improved peak shape leading to a choice of 1.2 mL/min as the optimum flow rate for both the 5 µm and 10 µm packed columns (Fig. 2.8). The backpressure of 2.54 kpsi in the 5 µm column prohibited the use of higher flow rates.

Flow Rate	Pressure	t_R (PPA)
(mL/min)	(kpsi)	(min.)
1.2	2.48	5.28
1.0	2.17	6.33
0.8	1.36	7.21
0.6	1.33	10.83

2.4.7 Peak Area and Peak Height Reproducibility

Good peak area and peak height reproducibility was obtained from replicate injections of a standard PPA.HCl solution. Reproducibility of the system is shown in Table 2.5 which shows retention time, peak area and peak height values from injections of the test mixture.

FIGURE 2.8 The effect of flow rate changes on the peak shape and separation of PPA (1) and EPH (2)

A) 0.6 mL/min

B) 1.2 mL/min

COLUMN: 5 µm Techsil

MOBILE PHASE: 23 % acetonitrile/77 % HSS solution (0.005 M) with 0.15 % 1 M HCl.

pH = 2.68

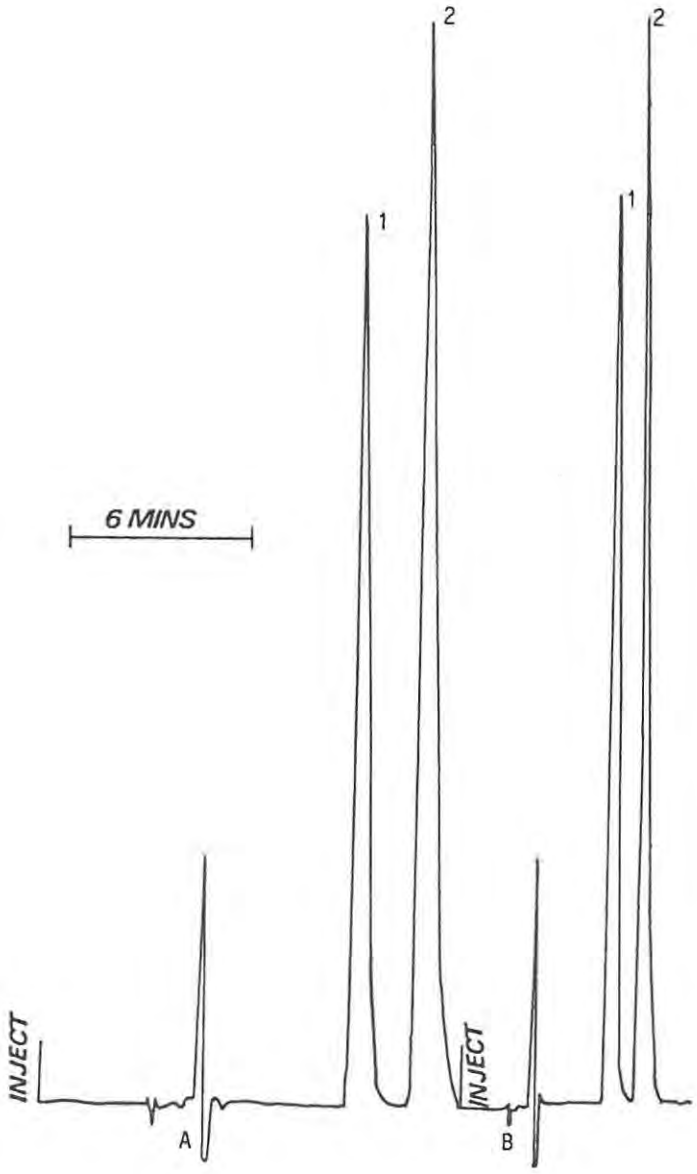


TABLE 2.5 Peak area and peak height reproducibility.

	Retention time (minutes)	Peak Area	Peak Height
1	5.16	3504	64936
2	5.16	3512	64951
3	5.16	3584	64934
4	5.16	3521	64962
5	5.16	3522	64939
6	5.16	3509	64973
7	5.16	3522	65001
8	5.16	3512	64932
9	5.16	3512	64935
10	5.16	3507	64941
11	5.16	3511	64931
12	5.17	3531	64965
13	5.17	3509	64955
14	5.17	3513	64954
15	5.17	3532	64959
16	5.17	3515	64932
17	5.17	3514	64931
18	5.17	3511	64948
19	5.17	3507	64942
20	5.17	3507	64936
MEAN	5.165	3517	64947
SD	0.005	17.37	17.83
R.S.D.	0.098%	2.49%	0.027%

CHAPTER 3

DETERMINATION OF PHENYLPROPANOLAMINE IN BIOLOGICAL FLUIDS

3.1 INTRODUCTION

Quantitative analysis of phenylpropanolamine in biological material is necessary for the investigation of pharmacokinetics and the correlation of drug levels with toxic effects. Since many of these studies are performed with very low doses, the requirements on determination limits are strong and may be as low as the lower nanogram range. Direct UV detection of PPA in assays presents problems because the molar absorptivity of PPA is very low (210). Hence, methods in which the drug has been derivatized or concentrated to increase specificity and sensitivity have been developed.

Using a periodate oxidation, Heimlich (40) developed a specific analytical technique for determining PPA in urine according to the original method of Shinn and Nicolet (213), in which PPA was converted to its corresponding aryl aldehyde. Derivative formation by his method affords a sensitivity 3-4 times greater than that for direct spectrophotometric determination of unconverted PPA. Periodate oxidation was also used by Chafetz (160) for the assay of PPA, but the procedure is not applicable to the analysis of the drug in biological specimens. In a procedure described by Wallace (162), phenylethanolamine drugs in biological specimens were determined by UV spectrophotometry after oxidation by means of alkaline periodate to benzylaldehyde. The sensitivity and specificity of the procedure was enhanced by subsequently converting the benzylaldehyde to the corresponding semicarbazone derivative. An early method for quantitatively determining PPA in plasma and urine was developed by Axelrod (35) involving extraction into benzene and assay by methyl orange reaction.

PPA in biological fluids has been assayed by thin-layer chromatography (TLC) following acetylation with tritiated acetic anhydride (214).

This method however suffers from a high coefficient of variation and nonlinearity. PPA has been separated and concentrated in urine by passing a buffered urine specimen through a column of Amberlite XAD-2 resin, a styrene divinylbenzene copolymer that adsorbs a wide variety of water soluble organic compounds, and then eluting the drug with an appropriate solvent which is evaporated. The residue is applied to thin layer plates, developed in a suitable solvent system and the drug detected qualitatively by sequential spraying (182, 215). After chloroform extraction of PPA from serum and urine, the drug has been reacted with 7-chloro-4-nitrobenzo-2oxa-1,3-diazole to produce a highly fluorescent compound which can be measured with a spectrofluorimeter (216). The extract is then evaporated and the residues subjected to thin-layer chromatography for qualitative identification of the drug.

The methods described above are time consuming in comparison to gas-liquid chromatography (GLC) for PPA in biological fluids. Kinsun *et al.* (217) described a quantitative determination of PPA in urine by GLC. After alkaline extraction with ether, PPA was analyzed directly by GLC without a derivatization step. For pharmacokinetic and bioavailability studies, PPA concentrations in biological fluids have also been determined by GLC following derivatization (180). Cummins and Fourier (218) used benzene to partially purify the serum after acid extraction followed by re-extraction with benzene after alkalinization and then formation of the heptafluorobutyryl derivative which was detected by gas chromatography with electron capture detection (GC-ECD). The method however is a tedious one as at least 4 hours are required for the derivative to form. Neelakantan and Kostenbauder (219) describe a GC-ECD procedure for the determination of PPA in plasma based on derivatization with pentafluorobenzaldehyde without prior extraction of the drug, to yield an electron-capturing derivative which is then readily extracted into hexane. The limits of sensitivity are 1.1 ng/3mL. A capillary GC analysis of PPA in human plasma after extraction with toluene from alkaline plasma, derivatization with trifluoroacetic anhydride and detection by electron capture

was presented in a recent report (220). Plasma concentrations above 5 ng/mL can be determined with accuracy and precision suitable for pharmacokinetic studies.

In the last few years, high-performance liquid chromotography (HPLC) has become a popular and widely used technique in the field of drug analysis from biological fluids. The methods used for treating biological samples prior to their introduction into an HPLC system generally fall into 2 categories - extraction or direct injection. In the extraction method, the compound of interest is removed from the biological matrix (plasma, serum, urine, etc) using a suitable solvent and pH to leave behind unwanted materials. The direct-injection technique is by far the simplest and most rapid of the two methods. However, this results in a rapid increase in back-pressure and deterioration of column performance (221, 222) due to precipitation of plasma proteins as a result of their contact with organic solvents and buffer salts commonly utilized in mobile phases.

An HPLC procedure described by Mason and Amick (223) involved extraction of PPA from basified plasma with 10% v/v 1-butanol and back extraction into a small volume of 1% acetic acid. This extract was derivatized with O-phthalaldehyde, and the derivative was measured using fluorescence detection to determine concentrations of PPA as low as 5 ng/mL. HPLC using detection in the visible range (450 nm) has been used to determine PPA in urine. Endo *et al.* (224) used a colour reaction with sodium β-naphthoquinone-4-sulphonate after extraction from large volumes of alkaline urine (50 mL) with hexane to determine PPA by HPLC. This method is time consuming and no indication is given as to the levels of PPA determined. Detection in the UV range resulted in interference from urinary constituents. Noggle (225) extracted PPA from alkaline urine with methylene chloride and then formed the phenylisocyanate derivative which was detected using HPLC at a wavelength of 254 nm. PPA has also been assayed in biological fluids by HPLC following extraction and pre-column derivatization with 4-chloro-7-nitrobenz-2,1,3 oxadiazole and sodium β-napthaquinone-4-sulphonate (226).

A method for the analysis of PPA in urine was developed by Dye and East (227) using HPLC and post-column in-line derivatization with O-phthalaldehyde followed by detection with a fluorometer. Human urine was injected directly onto the chromatographic system and PPA was separated in the reversed-phase mode. The limit of detection was 0.1 µg/mL of urine. An HPLC analysis of PPA in urine and plasma by post-column derivatization with O-phthalaldehyde has recently been described (228). Plasma samples were extracted with methylene chloride under alkaline conditions, and urine was diluted with mobile phase without extraction. Using fluorescence detection, the method is sufficiently sensitive (2 ng/mL of plasma and 0.5 µg/mL of urine) so that PPA concentrations could be measured up to 24 hours following a 75 mg oral dose.

The above mentioned HPLC methods used to determine PPA in biological fluids have involved derivatization methods which are relatively time consuming or involve post-column manipulations. Determination of PPA in serum and urine without prior derivatization has been described by Dowse *et al.* (211). The method involves extraction at a basic pH with chloroform, a single back extraction into acid and chromotography on a reverse-phase column. The detection limit for PPA was 25ng/mL in serum and 10 µg/mL in urine. The resulting chromotograms were clean with no interfering peaks due to endogenous constituents. An adaptation of this HPLC assay for PPA in serum was recently used to examine PPA pharmacokinetics in dogs (229). Plasma was extracted into aqueous acetic acid and injected onto a cyano column. Detection was by UV absorbance at 210 nm and the limit of detection was 30 ng/mL of plasma.

3.2 METHODS OF EXTRACTION OF PHENYLPROPANOLAMINE FROM SERUM

3.2.1 Experimental

3.2.1.1 Sample Preparation

Aqueous solutions of PPA.HCl (500.0 ng/mL) were prepared as in section 2.3.1.1. During these developmental stages, spiked serum samples of different concentrations were prepared by addition of the appropriate amounts of the aqueous solutions of PPA.HCl to drug free serum. The concentration ranges of serum varied between 12.5ng/mL and 500.0ng/mL.

3.2.1.2 Reagents

The sodium hydroxide[1], potassium hydroxide[2], sodium carbonate (anhydrous)[2], hydrochloric acid[2] and acetic acid[3] were obtained commercially. The methylene chloride[4] and chloroform[5] were HPLC grade.

1. Holpro Analytics (Pty) Ltd, H70106, Johannesburg, South Africa.
2. B.D.H Chemicals Ltd., Poole, England.
3. E. Merck, Darmstadt, West Germany.
4. Burdick and Jackson Laboratories, Muskegon, Mich., USA.
5. Waters Associates, Milford, Mass., USA.

3.2.1.3 Extraction Procedures

1.0 mL SPIKED SERUM + INTERNAL STANDARD (EPH.HCl) + BASIFYING AGENT

↓

VORTEX

↓

ADD ORGANIC SOLVENT

↓

VORTEX OR SLOW SHAKING

↓

CENTRIFUGE

↓

REMOVE AQUEOUS LAYER BY ASPIRATION

↓

TRANSFER ORGANIC EXTRACT TO TAPERED TUBE CONTAINING A SMALL VOLUME OF ACID (50-120 µL)

↓

VORTEX

↓

CENTRIFUGE

↓

REDUCE ORGANIC EXTRACT TO ABOUT 200 µL by ASPIRATION

↓

RE-CENTRIFUGE

↓

INJECT 15 TO 30 µL of the ACIDIC EXTRACT DIRECTLY ONTO THE COLUMN

The following extraction procedures using either chloroform or methylene chloride were assessed:

a) CHLOROFORM

I Chloroform/sodium carbonate/acetic acid

Aqueous solutions of PPA.HCl and for spiked serum samples were basified with 100 µL of a saturated sodium carbonate solution,

extracted by vortexing with varying volumes of chloroform (5, 8 and 10mL) and centrifuged. The lower layer was transferred to a tapered tube containing 5% v/v acetic acid and vortexed for 1 min to initiate back extraction. The chloroform layer was reduced by aspiration to about 200 µL and discarded. A 20 µL aliqout of the acetic acid extract was injected directly onto the column.

II Chloroform/sodium hydroxide/amyl alcohol/acetic acid

The serum sample was basified with 100 µL 10% NaOH, 10 drops of amyl alcohol were added and extracted with 8 mL chloroform. The tube was vortexed and centrifuged and the lower organic layer transferred to a tapered tube containing 100 µL of acetic acid (5% v/v). Ten to twenty microlitres of the acidic extract was injected onto the column.

III Chloroform/sodium hydroxide

This method was similar to that described in extraction I with 100 µL sodium hydroxide (10%) being used in place of sodium carbonate. The organic extracts were evaporated to dryness at 40°C under a gentle stream of nitrogen and reconstituted with 100 µL of mobile phase before direct injection of the sample onto the column.

IV Chloroform/sodium hydroxide/hydrochloric acid

Spiked serum samples were basified with varying concentrations of NaOH (2.5, 5.0 and 10.0% solutions). Chloroform (8 mL) was added and, after shaking, the organic phase was transferred to a tapered centrifuge tube containing 100 µL hydrochloric acid (0.1 M). Fifteen to thirty microlitres of the hydrochloric extract was injected directly onto the column.

b) METHYLENE CHLORIDE

V Methylene chloride/sodium carbonate/acetic acid

Spiked serum samples were treated as in extraction I, using methylene chloride (8 mL) in place of chloroform.

VI Methylene chloride/sodium carbonate/hydrochloric acid

The serum samples and aqueous PPA.HCl samples were basified with 100 µL sodium carbonate, extracted in 8 mL methylene chloride, and subsequently back-extracted into 100 µL hydrochloric acid (0.1 M). Twenty microlitres of the acidic extract was injected onto the column.

VII Methylene chloride/sodium hydroxide/hydrochloric acid

Spiked serum samples were treated as in extraction IV using methylene chloride (8 mL) in place of chloroform.

VIII Methylene chloride/potassium hydroxide/hydrochloric acid

A number of spiked serum samples were basified with 100 µL of either 0.5, 1.0, 2.5, 3.0, 5.0 or 10.0% KOH solutions. Methylene chloride (8 mL) was added, the tube vortexed for 20 sec and centrifuged. The lower layer was transferred to a tapered tube containing 100 µL hydrochloric acid (0.1 M) and a 20 µL aliqout was injected directly onto the column.

3.2.1.4 Chromatographic Conditions

The following chromatographic conditions were used after the extraction procedures I - III:

Column and Mobile phase:	2 (Table 2.1)
HPLC system:	C (Section 2.3.1.2)
Flow rate:	1.2 mL/min
Pressure and Temperature:	Table 2.1
Detection Wavelength:	210 nm
Attenuation:	0.01 AUFS
Recorder input:	10mV

For the remainder of the extraction procedures, the chromatographic conditions used were as follows:

Column and Mobile phase:	3 (Table 2.1)
HPLC system:	C (Section 2.3.1.2)
Flow rate:	1.2 mL/min
Pressure and Temperature:	Table 2.1
Detection Wavelength:	210 nm
Attenuation:	0.01 AUFS
Recorder input:	10mV

3.2.2 Results and Discussion

When trace amounts of therapeutic substances of comparatively low molecular mass have to be determined in serum or urine, an isolation procedure of variable complexity has to be applied initially (230). For pharmacokinetic interpretations, the analytical method must be sensitive, specific, accurate and precise, especially at the lower concentrations. The methods used for treating biological samples prior to their introduction into an HPLC system fall into one of two categories-extraction or direct injection. However, the latter method, although by far the simplest and most rapid, results in a rapid increase in back-pressure and deterioration of column performance (221). To alleviate this problem, a number of sample preparation techniques have been described for removing proteins prior to injection of the sample. These include the use of pre-columns (231), ultrafiltration devices (232) various protein precipitants such as organic solvents (233) and ionic salts (234), solvent extraction and selective adsorption of the drug onto a suitable substrate. Strong acids, such as trichloroacetic acid, perchloric acid or hydrochloric acid are frequently used to denature proteins giving a precipitate which can be separated by centrifugation. Protein precipitation by heavy metals, alcohol or dialysis is never 100% efficient, as the drug of interest often co-precipitates with the protein (235).

Drugs are most easily extracted from biological samples using water-immiscible solvents following the classical approach by Brodie *et al.* (236). Solvent extraction is generally the ideal method if a single primary extraction followed, if necessary by a back-extraction, provides a good recovery of the desired compound. Liquid-liquid

extraction is an isolation and separation method based on the distribution between two immiscible solvents that are in close contact with one another (158). Selective extraction with water-immiscible solvents relies upon the drug having a favourable partition coefficient, and the most common approach for protolytic compounds is to extract them in the uncharged form. Phenylpropanolamine, being a weak base with a pKa of 9.4 is most readily extracted by making it alkaline prior to its extraction into an organic solvent. Medium polarity solvents, such as chloroform and methylene chloride are suitable for extracting amines, which show much higher distribution in these solvents than the hydrogen-accepting solvents (158). The organic extract can then be further purified and concentrated by back-extraction or removal of the organic solvent at elevated temperatures under nitrogen. Care must be taken to ensure that the drug is not removed by evaporation, volatilization or lost during the extraction process.

Concentrations of PPA found in serum after the ingestion of doses of the drug between 25 and 100 mg range from 5 to 500 ng/mL. These low levels make sample extraction essential to enable direct UV detection of the drug.

Chloroform and methylene chloride (dichloromethane) are aliphatic chlorinated hydrocarbons that, on exposure to air and light, degrade to extremely reactive phosgene resulting in decomposition of the solvents. Commercially supplied chloroform contains a stabilizer such as ethanol, and the presence of ethyl chloroformate, has been shown to react with various drugs extracted with this solvent (237). General points that were taken into consideration with the solvent extraction procedure included solvent purity (238), inadvertant exposure of the solvent to air and light (239), cost and toxicity of the solvent.

The first solvent to be assessed was chloroform, the specific density of which can be a disadvantage in the manipulations since it is heavier than water and forms the lower layer when mixed. Chloroform has previously been used to extract PPA from body fluids (34).

Initially, chloroform appeared to be very promising as it yielded an 80% recovery from an aqueous solution (extraction I). Two problems with serum extraction did arise, however, one being concerned with the formation of a solid gelatinous emulsion intractable by centrifuging, a phenomenon which has been previously described (230). The other problem was a high serum blank which occurred. Interference resulted even after the injection of extremely low volumes of extract.

Although centrifugation is often the most successful method of breaking emulsions, a number of other techniques have been proposed, including successive freezing and thawing, filtration, touching with a glass rod, saturating the aqueous layer with salt or the addition of a small amount of alcohol. Addition of alcohol to some samples that happen to form emulsions is undesirable if reproducibility of extraction is to be maintained (240). Attempts were thus made to develop a method whereby emulsions are prevented from forming. The problem of emulsification can be avoided by less intensive mixing of the phases, and a major pitfall was in the shaking technique. Too vigorous shaking produced emulsions, which are known to occur between an alkaline aqueous phase and halogenated solvents. Although these emulsions could be broken by centrifugation and subsequent vortexing, this was found to be time consuming. Extraction carried out in tubes clamped near to the horizontal position and shaken longitudinally at 120 oscillations per minute for 15 minutes on a rocking table were favoured when chloroform was used as the extracting solvent, as this gentle tilting minimized the risk of emulsification.

A study was carried out to attempt to ascertain the optimum pH for extraction by adding varying amounts of NaOH to the serum (extractions IV and VI). It was found that a 10% NaOH solution gave the best extraction efficiency when methylene chloride was used as the extracting solvent. Conversely, the 2.5% NaOH solution gave better extraction efficiency than the higher concentrations of basifying agents added to serum samples when chloroform was used as the organic solvent. With the increase in concentration of NaOH from 2.5% to 10%, the tendency to form emulsions increased, and the use of a more

concentrated solution of NaOH (10%) did not have any advantageous effect on recovery. Extraction I, when basifying with Na_2CO_3 yielded a better recovery than when NaOH was used.

In extraction II, amyl alcohol was added in the hope that it would reduce emulsion formation. It did help in preventing the formation of intractable emulsions but a problem encountered with this method was the lack of reproducibility and poor recovery (50%). An increase in the organic/aqueous ratio tended to increase recovery to 60%, but interfering peaks were observed with blank serum extracts.

The problem encountered with extraction III was the lack of reproducibility and a poor extraction efficiency of 30%, which was traced to the evaporation and reconstitution steps. Loss of drug seemed to occur during evaporation, possibly through volatilization of the free base form (217). Adsorption to the glass may have prevented complete solution of the drug in the mobile phase on reconstitution. Attempts were therefore undertaken to eliminate the evaporation step as the resultant variation in analytical recovery reflected poor calibration lines and poor reproducibility.

It was necessary to concentrate PPA further to enable detection at the lower concentrations. A final purification and concentration step was effected by back-extraction into a small volume of acid. This was favoured over the evaporation step, as recovery was much more consistent. However, a problem encountered with the acetic acid (5% v/v) was the appearance of an extremely large, broad hump which eluted about 1 hour after injection of the final acidic extract. The large hump interfered with subsequent chromatograms making it difficult to automate the system. The initial assessment was that it may have been due to an impurity in the acetic acid or a component that was extracted by the acetic acid during the extraction procedure. The problem was overcome by using a 0.1 M solution of hydrochloric acid instead of the acetic acid and cleaner extracts were thereby obtained.

The final solvent assessed was methylene chloride. This organic solvent had the advantage over chloroform in the extraction of PPA from serum in that no persistent gelatinous emulsions were formed and clean extracts resulted.

However, extractions V and VI yielded relatively poor recoveries of about 60%. Attempts to increase recovery were four-fold: altering the basifying agent, increasing the volume of methylene chloride (organic/aqueous ratio), and finally assessment of multiple extractions and mixing techniques. Three basifying agents (Na_2CO_3, NaOH and KOH) were assessed and varying concentrations of each were added to the serum (extraction VI, VII, VIII). A 10% KOH solution (extraction VII) gave the highest recovery, and it was found that a pH of 13 proved to be satisfactory with regard to selectivity and efficiency of extraction into methylene chloride. The volume of methylene chloride was increased from 5 to 10 mL and improved recovery by about 10%. The effect of multiple extractions was investigated and a 75% recovery after a single extraction was increased to 80% on extracting the sample twice. However, the slight increase did not justify the extra number of manipulations involved. A short, not too vigorous extraction was found to be more successful in producing "clean" extracts than long, violent encounters with the extracting solvent. Centrifugation was found to be advantageous in forming distinct solvent layers, particularly when proteins were precipitated by the action of the organic solvent. Care was taken to separate the two layers and the top layer was easily removed by aspiration with a pasteur pipette.

It is well established that the precision of the quantitative data increases if an internal standard is included in the extraction procedure. The internal standard (EPH.HCl) was added to the samples before the work-up procedure started and subjected to the entire analytical procedure.

It was found that a large peak eluted at about 10 min and after a small volume of methylene chloride was injected onto the column, the peak reappeared at 10 min. It was thus concluded that this peak was due to the presence of a small percentage of methylene chloride dissolved in the hydrochloric acid extract which was then injected.

The hydrochloric acid (120 µL) remained on top of the methylene chloride and appeared as globules adhering to the walls of the tube. After back-extraction, the methylene chloride was removed down to about 200 µL and discarded, thus allowing the small volume of acid to form a single, continuous upper layer from which aliquots for injection could be removed. The final method used is described in section 3.3.1.3. The analytical method thus developed is a simple, rapid process that allows the analysis of large numbers of samples.

Analysis of pre-dose serum samples from 10 human subjects and several batches of pooled human serum presented no chromatographic peaks that interfered with the internal standard or PPA. A previous report (241) suggested that no significant metabolism of PPA occurs, so *in vivo* interference by metabolites was not a problem. The extraction procedure produced clean extracts and very clean chromatograms, and thus should be considered for other primary amines. Although the method was developed with the purpose of studying the pharmacokinetics of PPA in humans following oral administration of the drug, it was also found to be suitable for the analysis of pseudoephedrine in humans following oral administration of the drug. Phenylpropanolamine would be a suitable internal standard for the analysis of this compound. A chromatogram of a serum extract containing pseudoephedrine is depicted in Fig. 3.5. The retention time of pseudoephedrine was 6.0 min.

3.3 ANALYSIS OF PHENYLPROPANOLAMINE IN SERUM

3.3.1 Experimental

3.3.1.1 Sample Preparation

Serum stock solution

Twenty five milligrams of PPA.HCl was accurately weighed and dissolved in 50.0 mL water and 1.0 mL of this solution was diluted to 10.0 mL with water. One millilitre of this solution was made up to 100.0 mL with blank serum. The stock solution was agitated by slow mixing for 1 hour. The resultant concentration in serum was 500.0 ng/mL. Appropriate dilutions were then made, ranging from 12.5 to 500.0 ng/mL.

Internal standard stock solutions

Forty milligrams of EPH.HCl was dissolved in 1.0 litre of water to yield 40 µg/mL. This solution was again diluted to yield a concentration of 2 µg/mL and this was used as the internal standard solution.

The serum used to prepare the serum stock solution was obtained from our local hospital (Settlers' Hospital, Grahamstown, South Africa). It was dialysed against an isotonic NaCl solution at 4°C for 96 hours to remove dialysable contaminants before use.

3.3.1.2 Chromatographic Conditions

Column:	5 µm Techsil
HPLC system:	D (Section 2.3.1.2)
Flow rate:	1.2 mL/min
Pressure:	2.53 kpsi
Temperature:	30°C
Detection Wavelength:	210 nm
Attenuation:	0.001 AUFS
Mobile phase:	0.005 M HSS solution/acetonitrile (77/23) with 0.15% 1 M HCl pH = 2.65

3.3.1.3 Extraction Procedure

One millilitre of serum, 100 µL EPH.HCl solution (2 µg/mL) and 100 µL of a 10% potassium hydroxide solution, which adjusted the pH of the mixture to 12.3, were mixed and vortexed for 20 sec in a culture tube. Eight millilitres of methylene chloride were added, the tube stoppered, vortexed for 15 sec, and centrifuged at 3 500 rpm for 2 min. A further 2 mL of methylene chloride was added, and the tube was re-centrifuged at 3 500 rpm for 3 min. A disc formed between the aqueous and organic phases. The aqueous layer above this disc was removed by aspiration and discarded. A pasteur pipette was used to transfer the methylene chloride extract to a tapered centrifuged tube containing 120 µL of a 0.1 M HCl solution. Care was taken to ensure that no droplets of serum were transferred to the tapered tube. The centrifuged tube was vortexed for 30 sec and centrifuged at 1 500 rpm for 2 min. The methylene chloride layer was reduced by aspiration to about 200 µL and the tube was re-centrifuged for a further 2 min at 1 500 rpm.

Aliquots of 80-100 µL of the hydrochloric acid extract were transferred by syringe to limited volume glass inserts and inserted in the autosampler. Twenty microlitres of this extract was injected directly onto the column.

3.3.1.4 Calibration Curve

The serum stock solution (500.0 ng/mL) was diluted to yield six different concentrations, the stock solution providing the seventh. Concentrations ranged from 12.5 to 500.0 ng/mL and each concentration was assayed in triplicate. The calibration curve was constructed by plotting the ratio of the peak height of PPA to that of the internal standard, versus the respective PPA concentration. The data were fitted by least-squares linear regression analysis.

3.3.1.5 Precision

Within-run precision was assayed by extracting five spiked serum samples at each concentration (12.5, 25.0, 50.0, 100.0, 200.0, 300.0 and 500.0 ng/mL).

3.3.1.6 Extraction Efficiency

i) For this study, in which the analytical recovery of PPA from serum was assessed, spiked serum samples were assayed in quadruplicate at five different concentrations. A similar extraction procedure to the one described in section 3.3.1.3 was applied to all the samples with one modification. The internal standard was omitted at the start of the procedure, and instead, 100 µL water was added to the serum. The EPH.HCl was dissolved in the hydrochloric acid and was thus not carried through the extraction procedure.

Standard solutions of PPA.HCl were made up with the internal standard solution in concentrations corresponding to those extracted above. The internal standard therefore accounted for variations in sample volumes injected. To determine the percentage recovery, the ratios obtained from the serum extracts were compared to those resulting from the relevant standard solution of equivalent concentrations.

ii) Analytical recovery of PPA.HCl from serum was also assessed without the use of the internal standard, EPH.HCl. This could be carried out successfully with the use of the constant volume 20 µL loop attached to the autosampler, as there is negligible variation (0.01%) in sample volumes injected.

Spiked serum samples were assayed in quadruplicate at four different concentrations. A similar extraction procedure to the one described in i) above was applied to all samples with one modification. The internal standard was omitted throughout the procedure.

Standard aqueous solutions of PPA.HCl were made up in concentrations corresponding to those extracted above. To determine percentage recovery, the peak heights obtained from the serum extracts were compared to those resulting from relevant aqueous standard solutions of equivalent concentrations.

3.3.2 Results and Discussion

3.3.2.1 Linearity and Calibration

Linearity was established for the range of concentrations studied (12.5-500.0 ng/mL). The line had a slope of 0.005638, a y-intercept of 0.016339 and a correlation coefficient of 0.9977. The calibration curve for phenylpropanolamine in serum is shown in Fig. 3.1. Chromatograms of a blank serum extract and a serum extract containing phenylpropanolamine and internal standard are depicted in Fig 3.2. The retention time of PPA was 5.6 min and that of EPH was 6.5 min.

FIGURE 3.1 Calibration curve of phenylpropanolamine in serum.

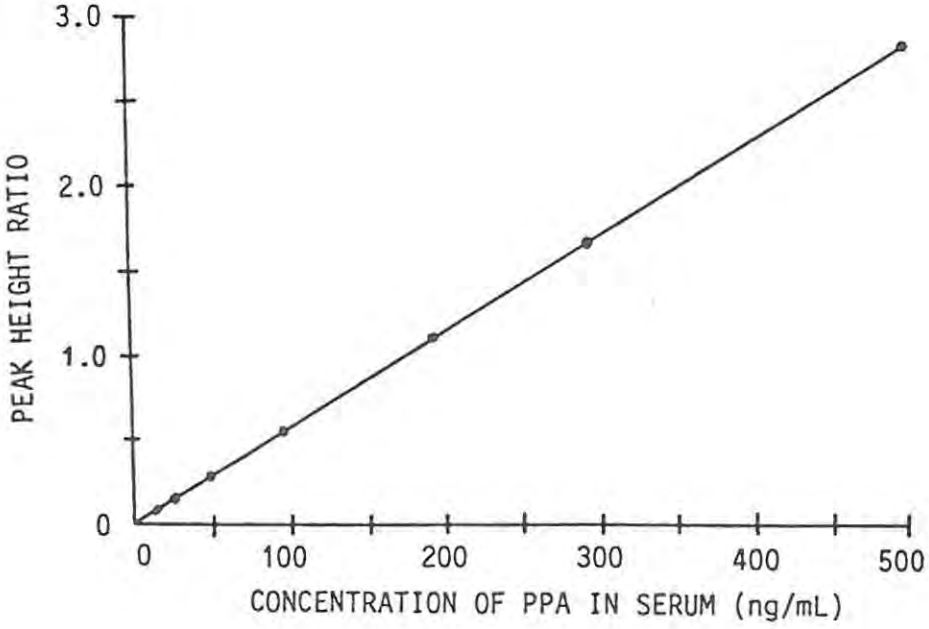
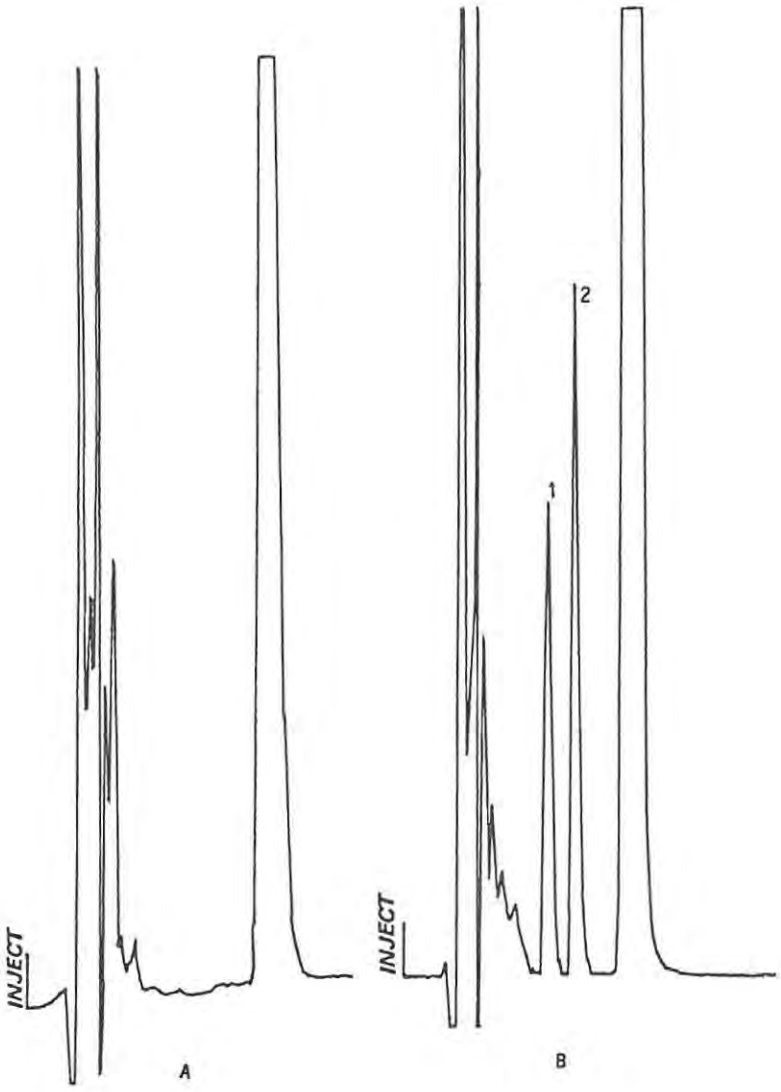


FIGURE 3.2 Chromatograms of (A) a blank serum extract and (B) a serum extract containing PPA (1) and EPH (2). The retention time of PPA was 5.6 min. and that of EPH was 6.5 min.



Column:	5 µm Techsil
Mobile Phase:	Acetonitrile/HSS solution (0.005 M) (23.5/76.5) with 0.2% 1 M HCl solution
Phenylpropanolamine concentration:	100 ng/mL

3.3.2.2 Precision

Results of the within-run precision study are depicted in Table 3.1. The method was found to be highly reproducible with R.S.D. values less than 10% at all concentrations.

3.3.2.3 Extraction Efficiency

Table 3.2 shows the analytical recoveries of PPA from serum. Results indicate that the recovery of PPA is constant and quantitative over the concentration range (12.5-500.0 ng/mL) studied. The mean recovery was 79.58%

TABLE 3.1 Within-run precision study on serum assay

Spiked conc (ng/mL)	Concentration measured (ng/mL) Sample number 1	2	3	4	5	Mean (SD)	RSD
12.5	13.79	13.79	12.85	13.23	13.79	13.48 (0.44)	3.25%
25	23.20	26.14	26.69	25.77	25.77	25.40 (1.34)	5.29%
50	53.04	50.00	49.82	49.10	49.10	50.79 (1.65)	3.25%
100	100.97	106.10	99.56	107.87	107.87	104.47 (3.94)	3.77%
200	209.06	187.99	207.30	189.74	205.55	199.93 (10.19)	5.10%
300	292.01	284.91	292.01	284.91	284.91	287.75 (3.89)	1.35%
500	491.76	490.01	535.50	523.10	523.10	505.03 (22.70)	4.50%

TABLE 3.2 Analytical recoveries of phenylpropanolamine from serum

Serum conc (ng/mL)	% Recovery Sample number 1	2	3	4	Mean (SD)	RSD
12.5	81.50	86.66	80.45	89.19	84.45 (4.16)	4.93%
50	75.00	80.55	81.11	80.55	79.30 (2.88)	3.63%
100	73.00	81.87	83.94	82.51	80.33 (4.96)	6.17%
250	73.77	71.85	77.81	71.78	73.80 (2.82)	3.82%
500	81.56	81.23	75.69	81.61	80.02 (2.89)	3.62%
Mean percentage recovery (n = 20)					79.58 (4.77)	5.98%

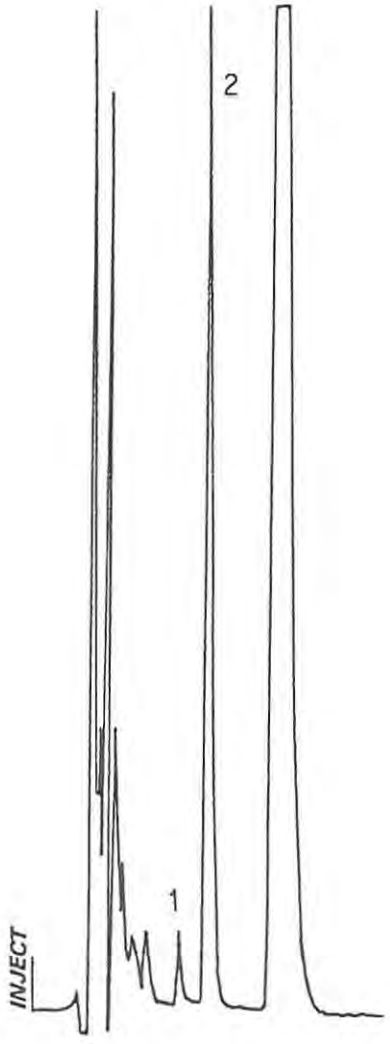
3.3.2.4 Sensitivity and Detection Limits

Under the conditions of this assay and based on a minimum signal to noise ratio of 10:1 the detection limit for phenylpropanolamine in serum was 5.0 ng/mL. A chromatogram of a serum extract containing PPA (5 ng/mL) is depicted in Fig. 3.2.1.

3.3.2.5 Background Interference

The technique of back-extraction eliminated a significant amount of interfering endogenous substances which had been co-extracted with methylene chloride. The hydrochloric acid eluted at the void volume and peaks from serum constituents did not interfere with the peaks of interest. The large peak at 9 min was due to a trace amount of methylene chloride which partitioned into the hydrochloric acid.

FIGURE 3.2.1 Chromatogram of a serum extract containing PPA (5 ng/mL) (1) and EPH (2).



Column: 5 µm Techsil

Mobile Phase: Acetonitrile/HSS solution (0.005 M) (23.5/76.5) with 0.2% 1 M HCl solution

3.3.2.6 Stability of Samples on Storage

Spiked serum samples (50, 250 and 500.0 ng/mL) were stored at -20°C and were assayed every 2 weeks over 2 months. The PPA content remained constant with the relative standard deviation falling within the acceptable limits i.e., less than 10% at all concentrations.

3.4 METHODS OF EXTRACTION OF PHENYLPROPANOLAMINE FROM URINE

3.4.1 Experimental

3.4.1.1 Sample Preparation

Spiked urine samples of different concentrations (10-100 µg/mL) were prepared as in Section 2.3.1.1 by the addition of appropriate amounts of aqueous solutions of PPA.HCl to freshly collected drug-free urine.

3.4.1.2 Extraction Procedure

Method I

Spiked urine (1.0 mL) was pipetted into a test tube and basified with 100 µL of a 10% NaOH solution. Five millilitres of chloroform were added, the tube was stoppered and vortexed for 30 sec and centrifuged at 3 500 rpm for 5 min. The organic extract was back-extracted into varying volumes of acetic acid (100 µL-500.0 µL), and a 20 µL aliquot of this extract was injected directly onto the column.

Method II

A modification of the final method used for serum was assessed (see extraction VIII on method development). The modification involved a smaller volume of methylene chloride (5-8 mL), and an increase in the volume of the 0.1 M hydrochloric acid solution used in the back-extraction (200 L or 500.0 µL instead of 100 µL).

3.4.1.3 Chromatographic Conditions

Column:	5 µm Techsil
HPLC system:	C, D (Section 2.3.1.2)
Flow rate:	1.2 mL/min
Pressure:	2.54 kpsi
Temperature:	30°C
Detection wavelength:	210 nm
Attenuation:	0.01 AUFS (Pye Unicam detector) or
	0.001 AUFS (Spectra-Physics detector)
Mobile phase:	0.005 M HSS solution /
	acetonitrile (77/23) with 0.15% 1 M HCl
	pH = 2.68

3.4.2 Results and Discussion

Urine contains numerous inorganic salts such as NaCl and also some organic endogenous compounds. The composition of urine varies much more than that of serum since it is influenced by intake of food and fluid. Typical urine contains upwards of 100 compounds (242) in concentrations that can readily be seen using a standard UV detector, therefore selective and effective isolation of the compound of interest is necessary.

The method developed for the serum analysis was successfully applied to the determination of PPA in urine with minor modifications (method II). Analysis of freshly collected urine samples from 10 human subjects presented no interfering chromatographic peaks. The final method used is simple, reproducible and yielded an analytically clean extract.

3.5 ANALYSIS OF PHENYLPROPANOLAMINE IN URINE

3.5.1 Experimental

3.5.1.1 Sample Preparation

Urine stock solution

Twenty milligrams PPA.HCl was dissolved in 100.0 mL blank urine to yield a solution of 200.0 µg/mL. Dilutions were made from this solution.

Internal standard stock solution

Forty milligrams EPH.HCl was dissolved in 100.0 mL water to yield a 400.0 µg/mL solution, which was then used as the internal standard solution.

3.5.1.2 Chromatographic Conditions

Column:	5 µm Techsil
HPLC system:	D (Section 2.3.1.2)
Flow rate:	1.2 mL/min
Pressure:	2.54 kpsi
Temperature:	30°C
Detection wavelength:	210 nm
Attenuation:	0.001 AUFS
Mobile phase:	0.005 M HSS solution/ acetonitrile (77/23) with 0.15% 1 M HCl pH = 2.6

3.5.1.3 Extraction Procedure

To 1 mL of urine in a test tube, 100 µL EPH.HCl aqueous solution (400 µg/mL) was added, and 100 µL of a 10% KOH solution to adjust the pH to 13.8. The tube was vortexed for 20 sec. Eight millilitres of methylene chloride was added and the tube stoppered and vortexed for 30 sec. The aqueous phase above was then removed by aspiration and discarded. A pasteur pipette was used to transfer the methylene chloride extract into a tapered centrifuge tube containing 200 µL 0.1 M HCl solution.

The tube was vortexed for 20 sec and centrifuged at 3 500 rpm for 1 min. The methylene chloride layer was reduced by aspiration to about 200 µL and discarded, and the tube was re-centrifuged for 5 min at 1 500 rpm. Aliquots of 80-100 µL of the hydrochloric acid extract were transferred to glass inserts for use in the autosampler and 20 µL of this was directly injected onto the column.

3.5.1.4 Calibration Curve

The urine stock solution was diluted with urine to yield different concentrations, with the stock solution providing the sixth. Concentrations ranged from 10.0 to 200.0 µg/mL and each concentration was determined in quadruplicate. A calibration curve was constructed.

3.5.1.5 Precision

Within-run precision was assessed in a similar manner to that described in section 3.3.1.

3.5.1.6 Extraction Efficiency

The method used for assessing extraction efficiency is described in section 3.3.1.6.

3.5.2 Results and Discussion

3.5.2.1 Linearity and Calibration

The calibration curve (see Fig. 3.3) was found to be linear over the range of concentrations studied (10.0-200.0 µg/mL) with a slope of 0.019919, a y-intercept of 0.024292 and a correlation coefficient of 0.9916. Relevant chromatograms are shown in Fig. 3.4.

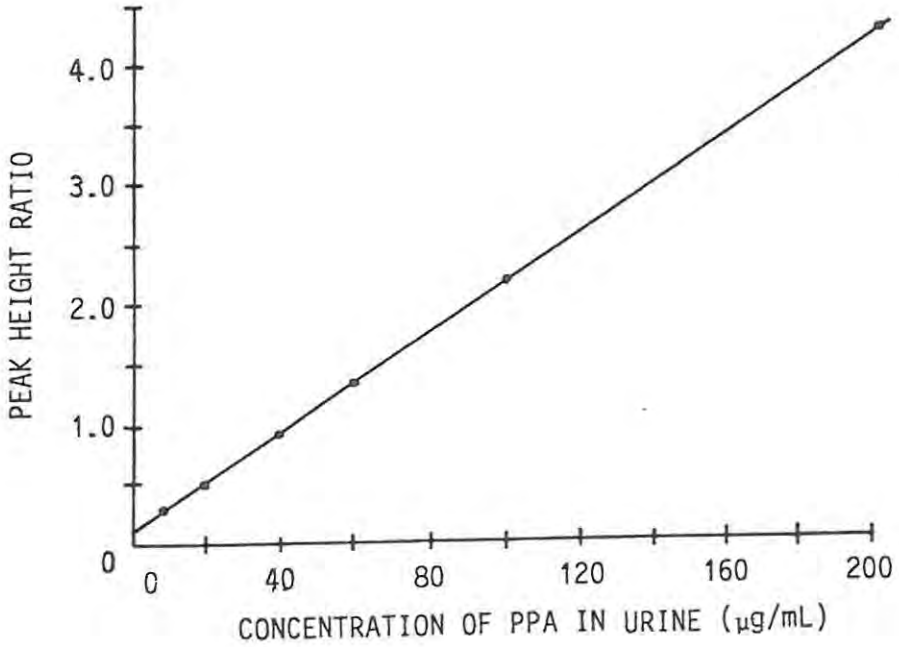
3.5.2.2 Precision

Results of the within-run precision study in urine are shown in Table 3.3.

TABLE 3.3 Within-run precision study on urine assay

Spiked conc (μg/mL)	Concentration measured (μg/mL) Sample number 1	2	3	4	5	6	Mean (SD)	RSD
200	188.94	185.58	194.05	184.50	205.83	206.30	194.2 (9.77)	5.03%
100	102.91	105.15	104.70	105.10			104.5 (1.05)	1.01%
60	64.29	65.01	68.40	66.34	65.96	66.67	66.11 (1.43)	2.16%
40	41.55	45.00	44.62	42.39			43.39 (1.68)	3.87%
20	17.91	18.75	18.03	17.97			18.17 (0.39)	2.16%
10	6.91	6.77	7.11	6.84	6.81	6.83	6.89 (0.12)	1.74%

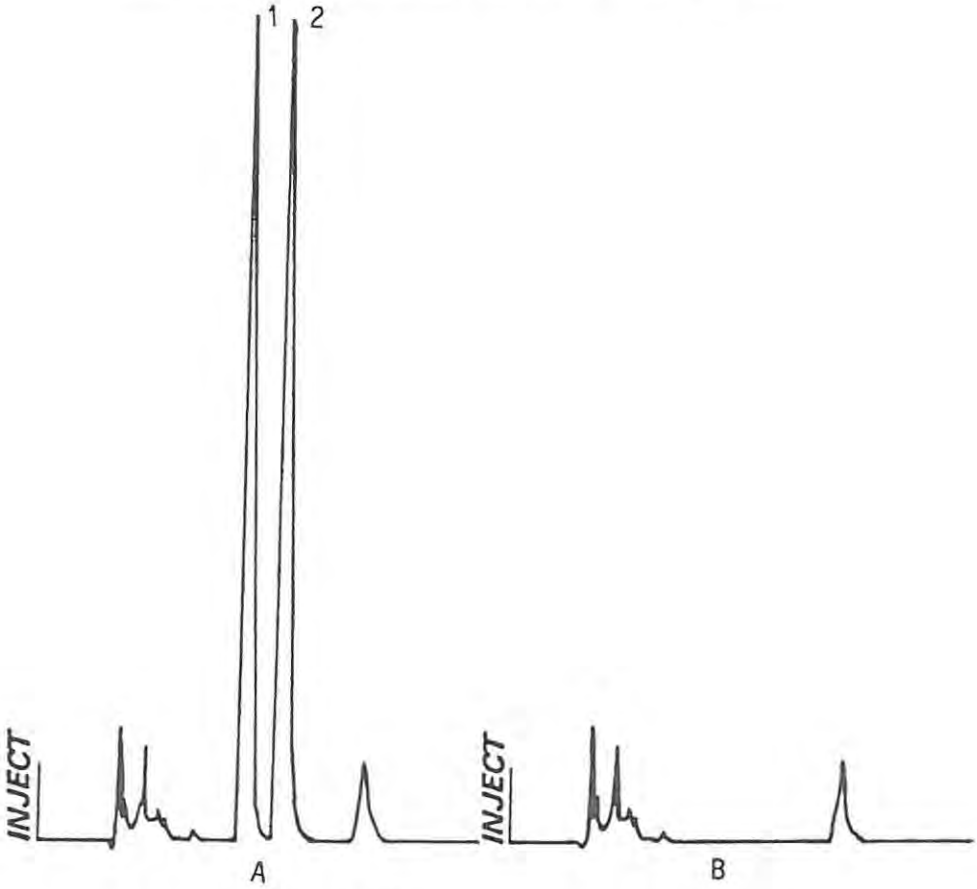
FIGURE 3.3 Calibration curve of phenylpropanolamine in urine.



3.5.2.3 Extraction Efficiency

The analytical recoveries of PPA from urine are shown in Table 3.4. The recoveries were constant over the entire concentration range studied, with a mean recovery of 83.49%.

FIGURE 3.4 Chromatograms of (A) a blank urine extract and (B) a urine extract containing PPA (1) and EPH (2).



Column: 5 µm Techsil

Mobile Phase: Acetonitrile/HSS solution (0.005 M) (23.5/76.5) with 0.2% 1 M HCl solution

TABLE 3.4 Analytical recoveries of phenylpropanolamine from urine

Urine conc (µg/mL)	% Recovery Sample number 1	2	3	4	5	Mean (SD)	RSD
10	74.39	76.58	78.29	75.37	77.32	76.39 (1.55)	2.02%
40	80.14	80.51	80.58	77.21	80.66	79.82 (1.47)	1.84%
60	80.00	83.24	78.92			80.72 (2.25)	2.79%
100	83.27	80.22	82.51	84.79	84.79	83.11 (1.90)	2.28%
200	95.34	96.98	96.43	96.43	96.41	96.30 (0.59)	0.62%
Mean % recovery (n=23)						83.49 (7.42)	8.88%

3.5.2.4 Sensitivity and Detection Limits

Under the conditions of this assay and based on a minimum signal to noise ratio of 10, the detection limit for PPA in urine was 5.0 µg/mL.

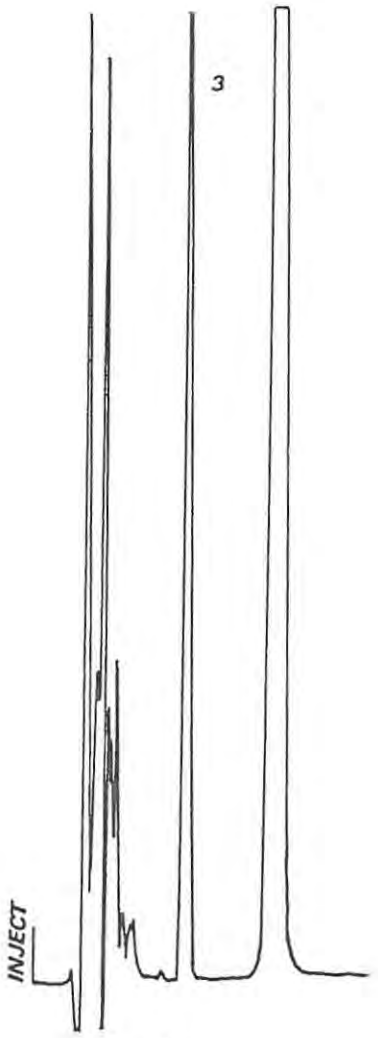
3.5.2.5 Background Interference

The hydrochloric acid and endogenous urinary constituents eluted near the void volume and no interference with the PPA or EPH peaks was encountered.

3.5.2.6 Stability of Samples on Storage

Spiked urine samples, stored at -20°C, were assayed over a 3 month period and were found to be constant with respect to PPA content, with relative standard deviations falling within the acceptable range i.e., less than 10% at all concentrations.

FIGURE 3.5 Chromatogram of (C) a serum extract containing pseudoephedrine (3). The retention time of pseudoephedrine was 6 min.



Column: 5 µm Techsil

Mobile Phase: Acetonitrile/HSS solution (0.005 M) (23.5/76.5) with 0.2% 1 M HCl solution

CHAPTER 4

THE *IN VIVO* EVALUATION OF PHENYLPROPANOLAMINE

In vivo trials on human volunteers were undertaken to enable the elucidation of the pharmacokinetic parameters of PPA. Although extensively used, there is relatively little pharmacokinetic information on PPA absorption and disposition.

4.1 PILOT TRIAL

A pilot trial using two male volunteers was conducted to establish various conditions such as frequency and times of sampling as well as the PPA concentrations which could be expected in serum and urine after the ingestion of a single 25 mg and 50 mg dose of the raw material in solution, and the necessity for monitoring blood pressure.

The volunteers (GB and JM) were non-smokers and had no previous history of hypertension. Individual details of the volunteers are given in Table 4.1. Blood and urine samples collected prior to the trial were subjected to clinical chemistry tests and were found to be normal. Each volunteer received a copy of the trial protocol and signed an informed consent form. They received an honorarium for participating in the trial and practised the same standardization procedures as described in section 4.2.4.

The test dose consisted of either 50 mg (subject JM) or 25 mg (subject GB) PPA.HCl dissolved in 200 mL water ingested at 0 hours. Blood samples were drawn prior to the trial as a blank and then at 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 5, 6, 8, 9, 12 and and 24 hours after ingestion of the initial dose of the drug. Urine was collected before the trial as a blank and at 2, 4, 6, 8, 12, 24 and then randomly collected up to 48 hours. Blood pressure readings were taken at 0, 4, 9, 12 and 24 hours after ingestion of the test dose.

The blood pressure readings seemed to indicate an increasing trend after ingestion of the drug, so blood pressure was monitored in subsequent trials to enable early detection of any possible dangerous rise in blood pressure. Hydralazine was kept available for any hypertensive emergencies.

4.2 CLINICAL TRIALS: METHODS AND DESIGN

To obtain meaningful data, randomized, controlled, crossover single dose and multiple dose studies were undertaken.

4.2.1 Volunteers

Five normal, healthy, caucasian adult male volunteers who were non-smokers were selected to participate in both the single dose and multiple dose studies.

The exclusion criteria were:

1) if they had received medication within four weeks preceding the initiation of this study or
2) required any concomitant medication on a daily basis;
3) had a known hypersensitivity to PPA.HCl;
4) had a history of serious cardiac, renal, hepatic or gastro-intestinal disease;
5) had an inability to communicate properly with the investigator.
6) Diabetes mellitus, asthma and/or treatment with antidepressant drugs.

Individual details of the volunteers are given in Table 4.1.

A complete medical history was taken and physical evaluation made at the time of the initial visit. The following haematological examinations, clinical chemistry and urinalysis were undertaken:

Haematology: haemoglobin, haematocrit, WBC, platelet count, differential count, RBC.

Blood chemistry: serum creatinine, serum urea, blood sugar, serum bilirubin, serum alkaline phosphatase, SGPT and SGOT.

Urinalysis: specific gravity, bile, pH, protein, glucose, RBC, WBC, epithelial cells, granular casts.

These parameters, namely physical examination, medical history, haematology tests, clinical chemistry tests and urinalysis were evaluated and subjects with abnormal values were not admitted into this study. All subjects were normal with systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg.

TABLE 4.1 Details of volunteers

Volunteer No.	Code Name	Age	Body Mass (kg)	Height (cm)
1	GB	20	59	178
2	WB	24	84	183
3	JM	20	75	188
4	AH	21	85	178.5
5	JS	23	61	183.5
Mean		21.6	72.4	182.2
± SD		1.82	12.91	4.10
R.S.D.		8.4%	17.8%	2.2%

All volunteers received a copy of the protocol, and written informed consent was obtained. The subjects selected indicated a willingness and good motivation to co-operate in this study. Volunteers were paid an honorarium for participating in the trial. All the test doses were compared in each subject.

4.2.2 Phenylpropanolamine Dosages

A = Phenylpropanolamine hydrochloride 25 mg

B = Phenylpropanolamine hydrochloride 50 mg

C = Phenylpropanolamine hydrochloride 100 mg

The raw material was dissolved in 200 mL water and ingested at zero time.

4.2.3 Treatments

TRIAL 1 - Single Dose Study

Subject Code Name	Phenylpropanolamine Dosage		
	Day 1	Day 8	Day 16
GB	C	B	A
WB	A	C	B
JM	A	C	B
AH	B	A	C
JS	B	A	C

Each trial subject received a single dose of either 25, 50 or 100 mg PPA.HCl powder dissolved in 200 mL water on the respective treatment days by random assignment. The washout period between study periods consisted of 7 days. The crossover was completed after each washout period with each subject receiving each treatment in the manner described above.

TRIAL 2 - Multiple Dose Study

Twenty five milligrams of the pure PPA.HCl powder (A), dissolved in 200 mL water was administered to each volunteer at 0, 4, 8, 12, 16, 20 and 24 hours.

4.2.4 Standardization Procedure

Identical test conditions were used for the treatment groups, as factors such as fasting and physical activity can influence drug absorption after oral administration.

1. The subjects received the study medication under close supervision while confined to the clinical unit. Medications were weighed out in individual beakers and coded by subject code and labelled with the appropriate study day.
2. Subjects were confined to bed for the first five hours of the trial, except when urine was voided; thereafter volunteers were allowed to move about freely and encouraged to resume normal activities, but had to refrain from engaging in any strenuous activities.
3. For the multiple dose study, each subject entered the clinical unit after the fifth dose of medication was administered, and remained confined to the unit until the end of the trial.
4. All volunteers had to conform to the following conditions:
   (a) No drugs, including over-the-counter preparations, were allowed for at least a week before the trial and for the duration of the trial.
   (b) No alcohol was consumed for at least 48 hours before the trial and for the duration of the trial.
   (c) No caffeine-containing food or drinks were ingested for at least 48 hours before the trial and for the duration of the trial. This included coffee, tea, chocolate and cola drinks.
   (d) No food or drink was ingested for 10 hours before the start of the trial up until the serving of a standardized breakfast two hours after taking the medication.
5. The standardized breakfast, consisted of 3 slices of toast with margarine and jam and 250 mL orange juice and was given 2 hours after the administration of the medication. A low-fat standardized lunch was given to all volunteers after the initiation of the study.

4.2.5 Sampling Schedules for the Trials

TRIAL 1 - Single Dose Study

The sampling procedures for the trials are given in Table 4.2.

TABLE 4.2 Sampling Schedule for Trials 1 and 2.

SAMPLING INTERVAL	TIME	TRIAL 1 and TRIAL 2		
		BLOOD	URINE	BLOOD PRESSURE
-10 min		B	U	BP
0[1]	8.00 am			
10 min	8.10 am	B		
20 min	8.20 am	B		
30 min	8.30 am	B		
40 min	8.40 am	B		BP
1 hr	9.00 am	B	U	
1.5 hr	9.30 am	B		BP
2 hr	10.00 am	B		
	BREAKFAST			
2.5 hr	10.30 am	B		BP
3 hr	11.00 am	B	U	
4 hr	12.00 noon	B		
5 hr	1.00 pm		U	
	LUNCH			
6 hr	2.00 pm	B		
7 hr	3.00 pm		U	BP
8 hr	4.00 pm	B		
9 hr	5.00 pm		U	
10 hr	6.00 pm	B		
11 hr	7.00 pm		U	
	SUPPER			
12.5 hr	8.30 pm	B		
14 hr	10.00 pm	B	U	
16 hr	12 midnight	B		BP

1 test dose ingested at this time

TRIAL 2 - Multiple Dose Study

After the seventh (24 hour) dose was administered the sampling schedule for the single dose study (Table 4.2) was followed. Before administration of the 1st, 4th, 5th and 6th doses (12, 16 and 20 hour respectively) blood samples were obtained to obtain PPA serum trough levels i.e. venipunctures were done at varying intervals before the subsequent dose was given, and just before the 1st dose was administered to obtain a serum blank.

4.2.6 Collection and Storage of Blood Samples

An indwelling 0.8 mm butterfly catheter (21 G, Medispo (Pty) Ltd., Industria, South Africa) was inserted into a suitable vein in the forearm and securely strapped into position with Micropore surgical tape (3M Medical Products Division, Johannesburg, South Africa) to allow complete mobility of the arm.

A 10 mL blood sample was withdrawn from the butterfly through a sterile hypodermic needle (0.8 x 40 LB, Medispo (Pty) Ltd., South Africa) by syringe aspiration. The butterfly was then flushed with 2 mL of sterile saline solution containing heparin (50 u/mL).

Immediately prior to the withdrawal of a further blood sample, the butterfly was cleared of the heparin solution by withdrawal of 2 mL of blood which was discarded. In a fresh syringe, 10 mL of blood was collected and transferred to a labelled vacutainer tube. The tube was stoppered and allowed to stand for 20 minutes for the blood to clot. Serum was isolated through centrifugation at 3 000 rpm for 20 minutes. One and a half millilitres of serum was transferred to a clean labelled vacutainer tube for analysis. The remaining serum was transferred into a second labelled vacutainer for storage. In a few cases, the serum contained fibrin clots, which were squeezed with the aid of an orange stick to free the serum. These samples were re-centrifuged to separate the serum. Duplicate serum samples were each labelled with a five-digit number. The serum samples were stored at -20°C for a maximum of 2 weeks until analysis. Spiked serum samples were stored frozen for a maximum of one month until the assays were performed.

4.2.7 Collection and Storage of Urine Samples

The collection and accurate recording of details were the responsibility of each volunteer and were not subject to the same rigid control as the collection of blood samples. The importance of strict adherence to sampling times and accurate recording of volume was thus stressed verbally in the protocol.

Volunteers were asked, for each sampling time, to void urine into 500 mL measuring cylinders, emptying the bladder as completely as possible each time. The total volume was measured by both the subject and co-ordinator before a representative sample of about 10 mL was transferred to a labelled glass vacutainer tube for storage, and a 1.5 mL sample transferred to a second vacutainer tube for analysis. The rest of the urine was discarded. Urine pH readings were taken immediately after collection and then frozen and stored at -20°C for a maximum of 2 weeks before analysis. Spiked urine samples were kept frozen for up to 6 months before analysis and no degradation of drug was observed. All drug samples were stored under identical conditions.

Urine voided at times other than those specified in the protocol were collected in the same way as above. The exact time of voiding was recorded, as well as total volume.

4.2.8 Blood Pressure Readings

Supine blood pressure readings were recorded by cuff and ausculation using a standardized mercury sphygmomanometer. Blood pressure was recorded at 0, 0.667 (40 min), 1.5, 2.5, 4, 8 and 16 hours after dosing.

4.2.9 Reporting of Side Effects

Patients who entered the study were asked to report any side effects or minor complaints throughout the course of the trial, and these were noted.

4.3 ANALYSIS OF BIOLOGICAL SAMPLES

4.3.1 The Analysis of Serum

Serum samples were analyzed in randomized order to avoid sequential effects. The aliquots (1.5 mL) were brought to room temperature and mixed thoroughly on a vortex mixer before being analyzed. The method of sample preparation and chromatographic conditions are described in section 3.3. Twenty microlitres of the final extract was injected onto the column. Both *in vitro* and *ex vivo* serum standards were prepared and analyzed under blinded conditions to monitor reproducibility of the method. All serum samples obtained for the test and reference preparations were stored under identical conditions and assayed by the same method.

4.3.2 The Analysis of Urine

Urine samples were brought to room temperature and mixed as in section 4.3.1. Each urine sample was analyzed according to the method described in section 3.3 and 20 microlitres of the final extract was injected into the column. Urine test samples, *ex vivo* and *in vitro* samples were analyzed in duplicate in a randomized order and all samples were assayed under identical conditions.

4.3.3 *In vitro* Quality Control for Phenylpropanolamine

The performance of the assay procedure was continuously monitored during the period of assay of the biological samples by including quality control specimens containing known concentrations of the analyte. These *in vitro* quality control samples were stored under identical conditions to those applying to actual trial samples.

4.3.4 *Ex vivo* Quality Control for Phenylpropanolamine

Additional blood was obtained from all trial subjects at predetermined times during Trials 1 and 2 and each serum or urine sample was divided into five aliquots. After analyzing one aliquot of each sample, eight that contained the analyte at concentrations spanning the expected range, were selected and re-analyzed at different times during the course of the analytical study. These *ex vivo* quality

control samples were stored under identical conditions to those applying to actual trial samples.

4.4 RESULTS

4.4.1 Side Effects

Side effects were reported by 4 of the volunteers after ingestion of the 100 mg PPA dose, and by 3 volunteers after ingestion of the 50 mg dose. No side effects were noted after the 25 mg PPA dose. Several relatively minor complaints were reported (Table 4.3). All symptoms disappeared when blood pressure returned to normal.

TABLE 4.3 Side Effects (All Patients)

	PPA 25 mg	PPA 50 mg	PPA 100 mg
Central Nervous System			
headache	0	0	1
anxiety	0	2	3
tiredness/lightheadedness	0	2	4
Autonomic Nervous System			
dry mouth	0	3	3
Gastrointestinal			
heartburn/epigastric pain	0	1	0
nausea	0	3	3
cramps	0	1	0
Genito-urinary			
frequent urination	0	1	2
Miscellaneous			
awareness of heart beat	0	2	3

Some patients reported more than one complaint

4.4.2 Blood Pressure Response to Phenylpropanolamine

Since this study was designed primarily to measure serum levels of PPA no placebo group was included.

Table 4.4 Blood pressure response to PPA

Dose PPA (mg)	Baseline BP (mmHg) (Syst/Diast)	Maximum BP (mm Hg) (Syst/Diast)	Change BP (mm Hg) (Syst/Diast)
25	121 ± 6/77 ± 7	120 ± 10/74 ± 9	1 ± 2/-3 ± 5
50	114 ± 4/78 ± 13	139 ± 17/89 ± 12	25 ± 1/11 ± 7
100	116 ± 12/80 ± 4	146 ± 16/94 ± 9	30 ± 1/14 ± 8

Data are $\bar{x} \pm SD$

n = 5 BP = Blood Pressure, Syst = systolic, Diast = diastolic

Table 4.5 Mean supine blood pressures and mean PPA serum levels

Hour	PPA: 25 mg BP (mmHg) (Syst/Diast)	PPA: 25 mg Serum level (ng/mL)	PPA: 50 mg BP (mmHg) (Syst/Diast)	PPA: 50 mg Serum level (ng/mL)	PPA: 100 mg BP (mmHg) (Syst/Diast)	PPA: 100 mg Serum level (ng/mL)
0	121/77	0.0	114/78	0.0	116/80	0.0
0.66	114/72	92.7	120/85	130.5	136/88	288.4
1.5	120/73	95.2	127/83	172.7	146/92	385.3
2.5	109/68	78.5	134/84	148.1	132/79	329.3
4	110/66	58.3	118/68	112.9	122/80	249.9
8	110/70	31.7	120/80	60.8	120/81	139.5
16	120/75	8.3	117/80	15.5	120/80	31.9

n = 5 BP = Blood Pressure, Syst = systolic, Diast = diastolic

Phenylpropanolamine increased blood pressure in a dose related fashion (Table 4.4). The highest blood pressure reading after 100 mg PPA was 160 mmHg systolic and 89 mmHg diastolic. Maximum increases occurred between 1 and 3 hours after PPA. Both systolic and diastolic blood pressures increased 90 minutes after 100 mg PPA values (Table 4.5). High serum levels of PPA appear to produce predictable responses in blood pressure. Patients receiving PPA showed small increases in mean systolic and diastolic blood pressure compared with baseline values (Table 4.4). Changes in mean blood pressure in the multiple dose study were found to be insignificant. Although there have been reports in recent years of adverse haemodynamic reactions to PPA (refer to section 1.5), our findings found that changes in mean blood pressure were clinically insignificant.

The variability of blood pressure readings reported throughout the test day is not unexpected. Pickering *et al.* (243) found that normal subjects had varied responses to different situations, with diastolic pressures ranging from 64 mm Hg for sleep to a high of 81 mm Hg for work.

PPA increased blood pressure with significant inter-subject variability. This inter-subject variability in the blood pressure response has been documented (83), although the reason for such variability is unknown and of great interest. Differences in pharmacokinetic parameters such as absorption, bioavailability, serum concentration dependency and receptor sensitivity have been proposed to explain this variability. American studies, which use the racemic mixture of PPA, have found either significant blood pressure elevations (85, 244) or no alteration (80, 245). The studies support marked inter-subject variability in blood pressure response to PPA similar to that demonstrated in this study. Individual blood pressure readings are listed in Table A 4.2.

4.4.3 Clinical Trial

The serum and urine concentrations from Trial 1 and 2 are presented in Tables A1.1 - A1.6 and the curves from Trial 1 depicted in Figs. 4.2-4.6. The mean serum concentration and urinary excretion profiles from the 5 subjects after the ingestion of a 25 mg, 50 mg or 100 mg PPA.HCl solution are shown in Figs. 4.7-4.9. The concentrations were within the quantitative range of the described method. The results obtained from the multiple dose study (Trial 2) are presented in Tables A 1.7 - A 1.8. Serum concentrations and urinary excretion profiles after the ingestion of a 25 mg PPA.HCl solution at 12, 16, 20 and 24 hours after the first dose was administered are depicted in Figs. 4.10-4.14.

In the 25 mg single dose solution study, absorption of the drug was rapid with peak blood concentrations occurring 40 minutes to 1.5 hours after ingestion of the solution and the concentrations ranged between 69 and 185 ng/mL of serum. Subject JM exhibited an extremely rapid absorption rate with a serum concentration of 185 ng/mL after 40

minutes, whereas the mean concentration of the other 4 subjects was 69 ng/mL at the same time. This discrepancy could be attributed to individual variability. In a study reported by Mason and Amick (223), the mean peak level occurred 2 hours after ingestion of a 25 mg PPA.HCl solution. After 16 hours, serum concentrations of the 25 mg solution study could not be accurately quantitated since they were found to be below 5 ng/mL.

Absorption of the drug in the 50 mg single dose solution study occurred 30 minutes to 1.5 hours after ingestion of the test dose and concentrations ranged between 149 and 207 ng/mL. In the 100 mg PPA.HCl solution study, peak concentrations occurred 1 to 2.5 hours after ingestion and ranged between 308 and 480 ng/mL. Two subjects, AH and JM exhibited more rapid absorption rates compared to the other subjects in the 25 mg, 50 mg and 100 mg PPA.HCL solution study. Conversely the absorption for subject WB was 1 hour slower for the 100 mg solution study, and for subject AH absorption was slower as the dose increased.

In the multiple dose study, serum concentration trough values varied between 72 and 130 ng/mL. Subjects JM and AH again displayed very rapid absorption with peak serum concentrations occurring 30 minutes after ingestion of the final test dose. This rapid absorption in these 2 subjects compares with that in Trial 1. Maximum serum concentrations of the multiple dose study were higher than those of the single dose 25 mg study and had a fairly narrow range (168-198 ng/mL).

Recovery of PPA from the urine after ingestion of 25 mg and 50 mg PPA.HCl was monitored for 24 hours in the pilot trial. An insignificant amount of PPA was found in the urine after 14 hours, and thus PPA was monitored for 14 hours in all subsequent trials. Sinsheimer *et al.* (241, 246) reported that 90% of the 25 mg oral dose was excreted in the urine as unchanged PPA, while in another report (247) 80-90% of the dose was excreted unchanged within 24 hours. Mean urinary recovery for the 5 subjects was:

a) 25 mg dose: 64.03%
b) 50 mg dose: 63.30%
c) 100 mg dose: 72.98%

Recovery could have been greater if the urine had been collected for a longer time as PPA was still clearly detectable in the blood after 16 hours. Urinary excretion of PPA in the 5 subjects after multiple dosing of 25 mg PPA.HCl was 68 mg, 67 mg, 73 mg, 60 mg and 61 mg respectively. Urine pH readings are listed in Table A 4.1. Biphasic serum concentration curves were observed repeatedly in a few subjects (WB, AH, GB) at different dose levels. This phenomenon has been discussed by Benet (248) and a physiological meaningful explanation can be given, when the possibility is considered, that the actual data are not concentration data of one compartment alone. A possibility mentioned was some kind of recycling mechanism other than biliary recycling (e.g. via the lymph system).

The method of blood withdrawal using butterfly sets was convenient as relatively little pain or trauma occurred in the initial insertion of the needle and after securing the butterfly with tape, the arm could be moved freely. Less trauma is associated with this method than with multiple venipuncture.

Table 4.6 details the inter-day precision of the method at three different serum concentrations using spiked *in vitro* serum standards. In Table 4.7, the inter-day and precision at three different urine concentrations using spiked *in vitro* urine standards are presented. The *ex vivo* urine and serum samples assessed during each run are depicted graphically in Fig 4.1. Average R.S.D.s for the method were less than 10% at all concentrations.

The method of extraction and subsequent determination of PPA from serum and urine is sensitive and precise. No derivatization is necessary, and the extraction was relatively simple with the back-extraction step eliminating the need for evaporation of the organic solvent, thus decreasing total sample preparation time significantly.

Concentrations of PPA following a single oral solution dose can be determined, which demonstrated that the method is adequate for use in bioavailability studies.

TABLE 4.6 Inter-day precision for phenylpropanolamine in serum

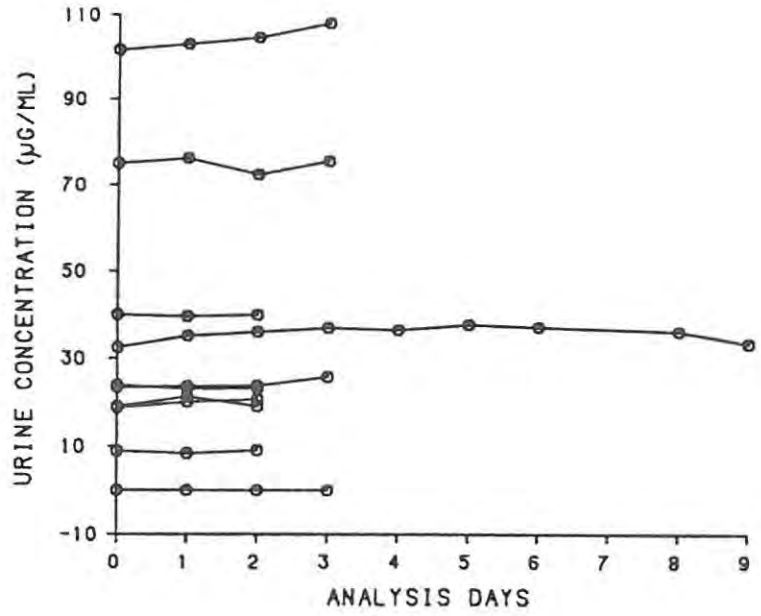
1	2	3	4	5	6	Mean (SD)	RSD
High concentration (spiked: 548.4 ng/mL)							
518.5	544.9	514.4	570.7	568.7	550.1	544.55 (24.02)	4.41%
Medium concentration (spiked: 219.4 ng/mL)							
229.9	200.4	198.6	247.6	218.5	214.9	218.32 (18.51)	8.48%
Low concentration (spiked: 25.12 ng/mL)							
21.9	24.2	26.8	28.8	25.5	25.2	25.41 (2.323)	9.14%

TABLE 4.7 Inter-day precision for phenylpropanolamine in urine

1	2	3	4	5	6	Mean (SD)	RSD
High concentration (spiked: 128.6 μg/mL)							
113.92	109.4	122.20	109.70	115.20	126.90	116.22 (7.00)	6.02%
Medium concentration (spiked: 51.44 μg/mL)							
53.36	58.47	53.02	49.36	55.20	43.76	52.2 (5.09)	9.76%
Low concentration (spiked: 10.28 μg/mL)							
8.55	7.80	7.80	9.76	11.06	9.02	8.998 (1.257)	13.97%

FIGURE 4.1 *In vivo* urine (a) and serum (b) samples assessed during each run.

(a)



(b)

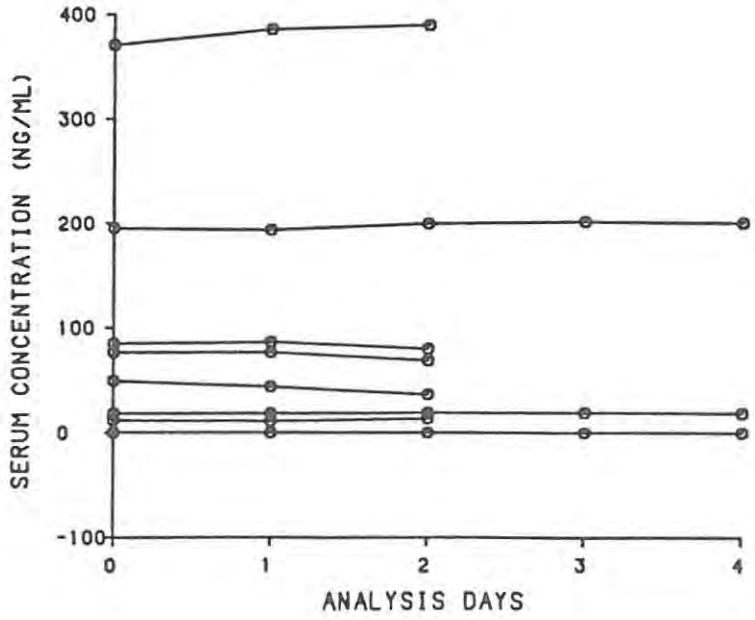
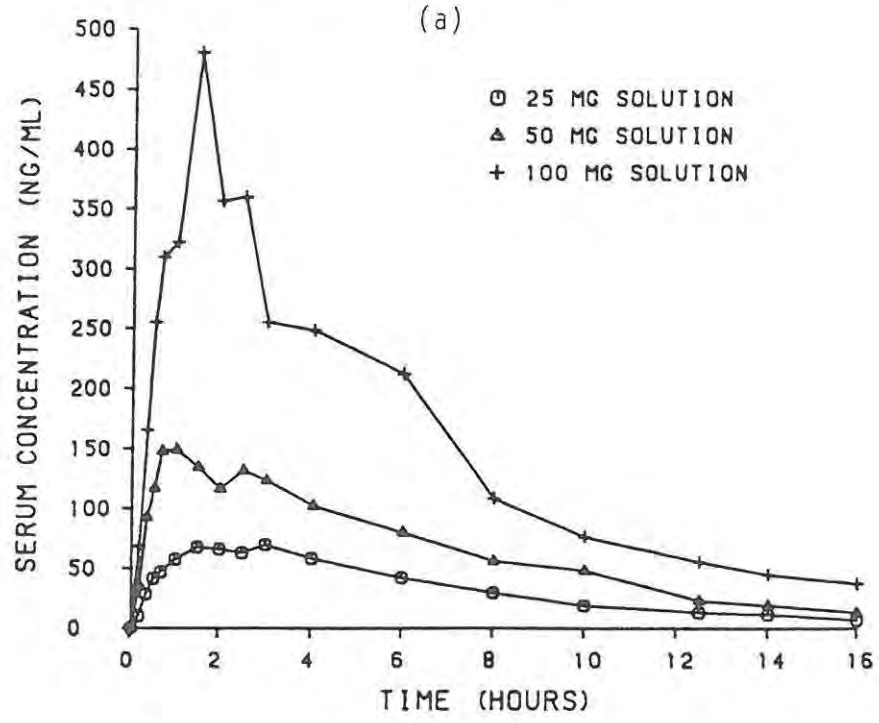


FIGURE 4.2 Serum concentrations (a) and urinary excretion profiles (b) from Subject GB after the ingestion of 25, 50 and 100 mg PPA.HCl solution.



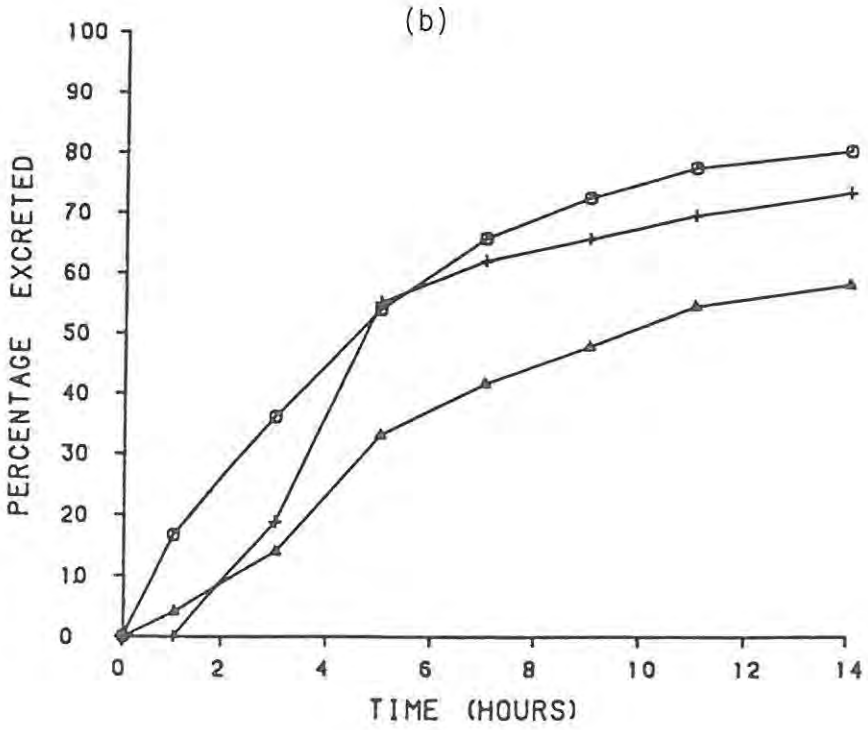
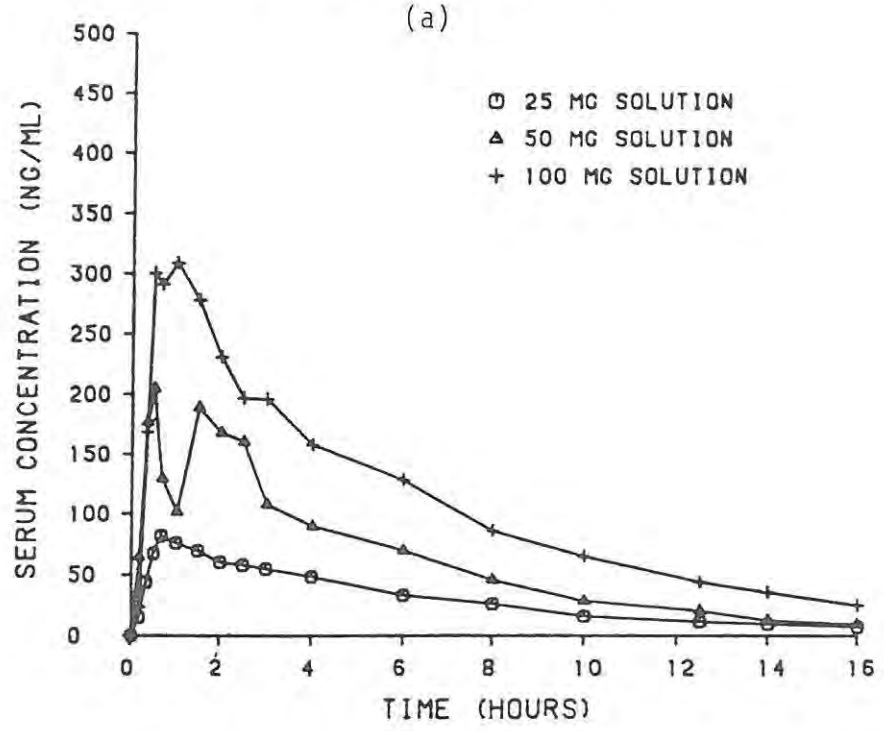


FIGURE 4.3 Serum concentrations (a) and urinary excretion profiles (b) from Subject AH after the ingestion of 25, 50 and 100 mg PPA.HCl solution.



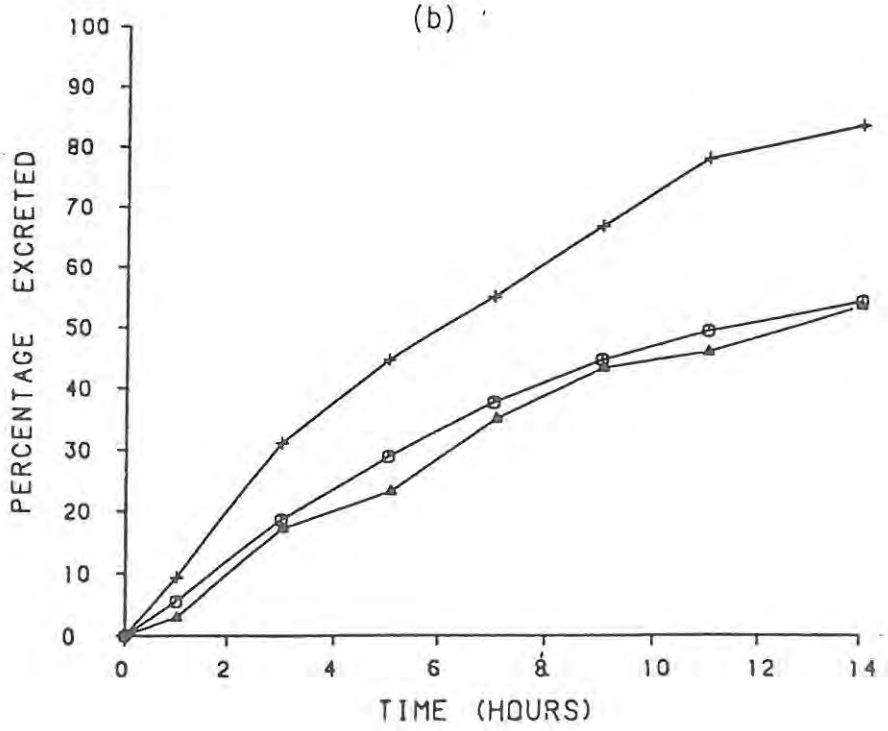
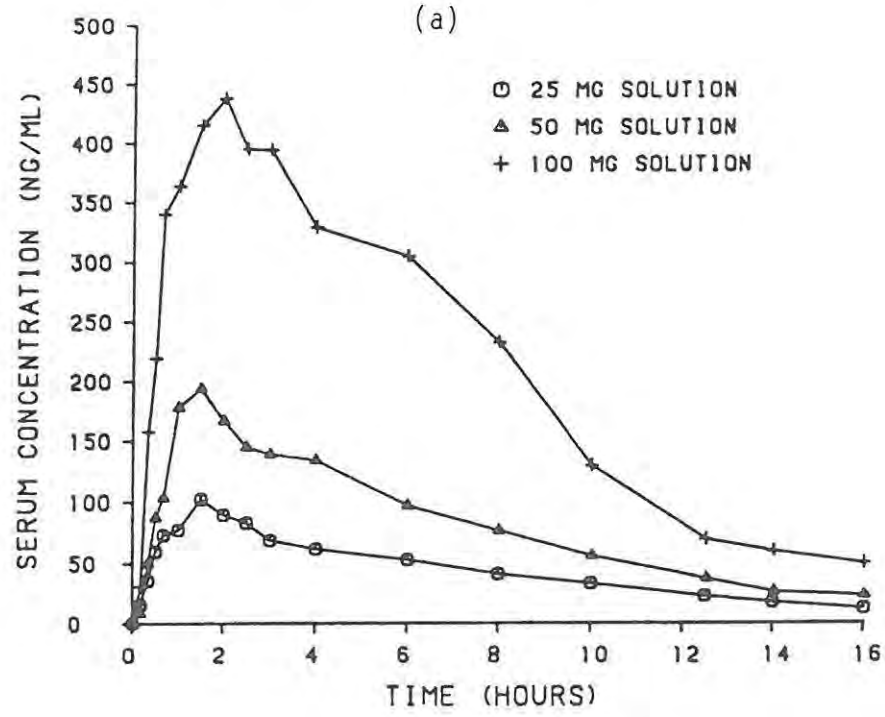


FIGURE 4.4 Serum concentrations (a) and urinary excretion profiles (b) from Subject JS after the ingestion of 25, 50 and 100 mg PPA.HCl solution.



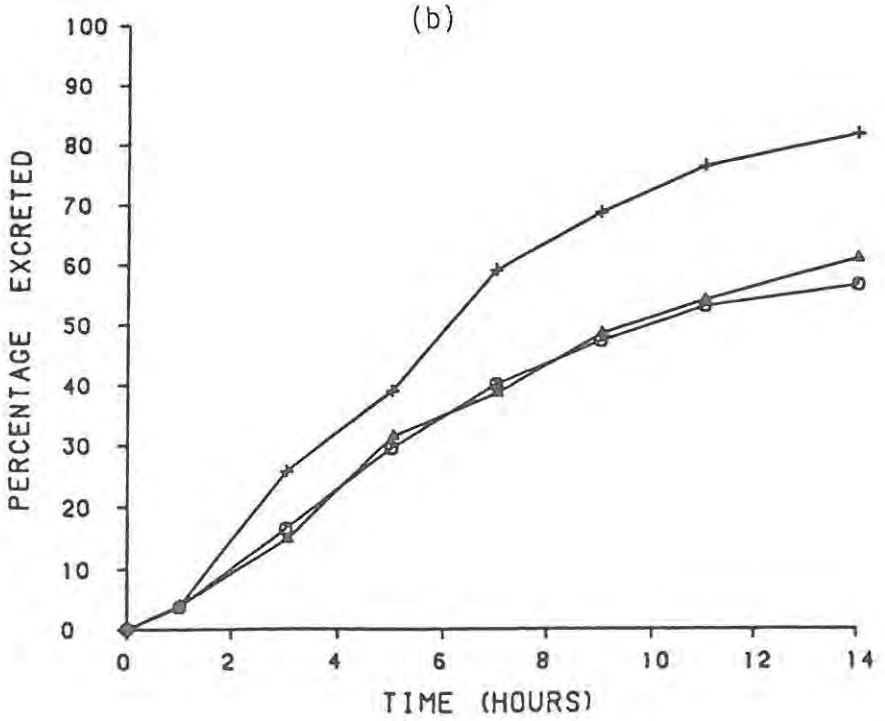
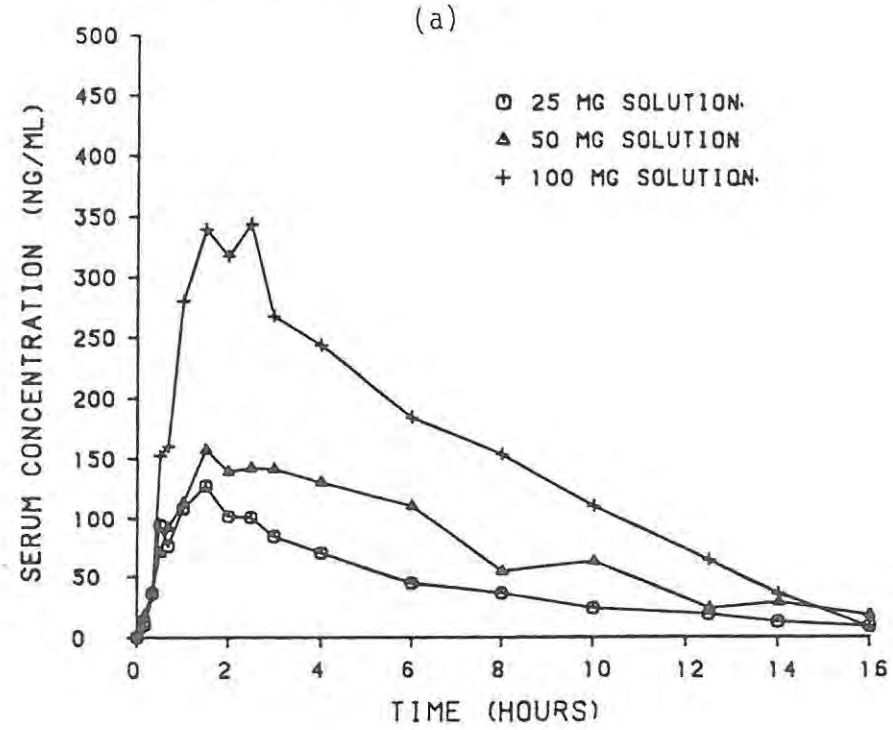


FIGURE 4.5 Serum concentrations (a) and urinary excretion profiles (b) from Subject WB after the ingestion of 25, 50 and 100 mg PPA.HCl solution.



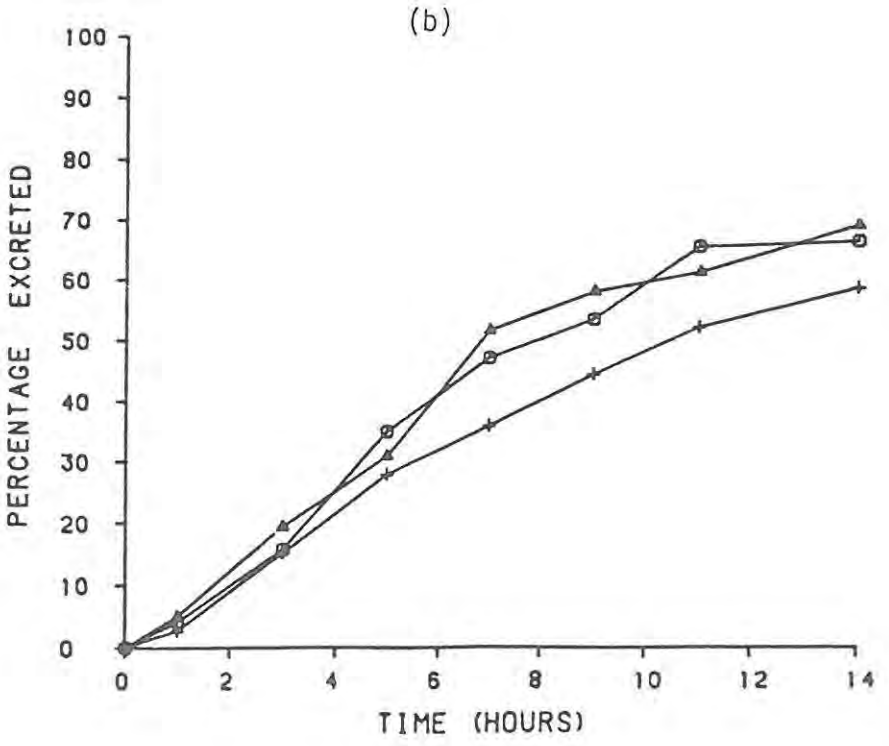
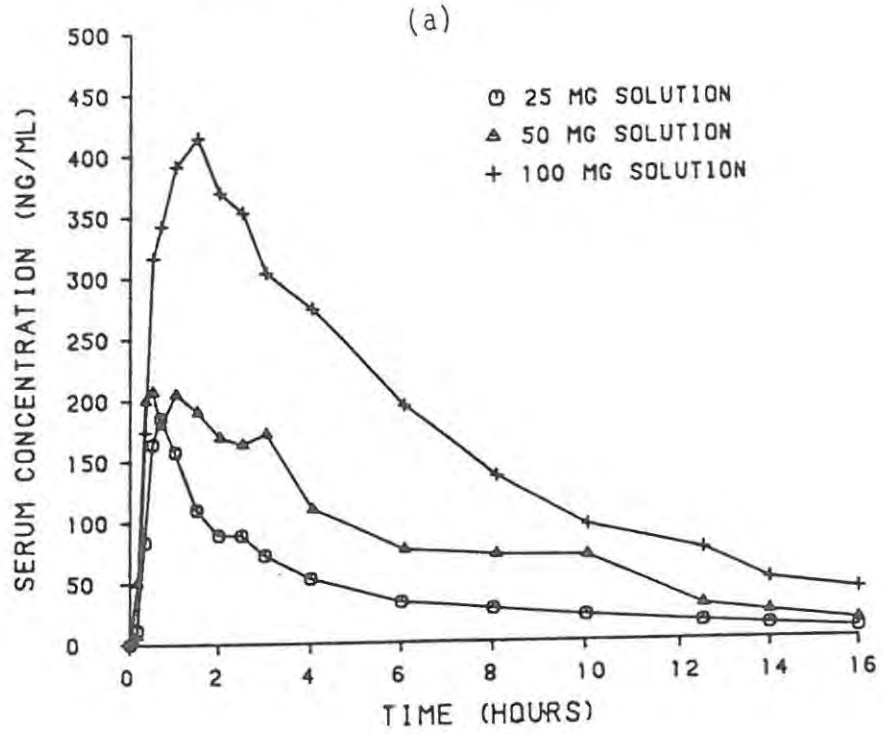


FIGURE 4.6 Serum concentrations (a) and urinary excretion profiles (b) from Subject JM after the ingestion of 25, 50 and 100 mg PPA.HCl solution.



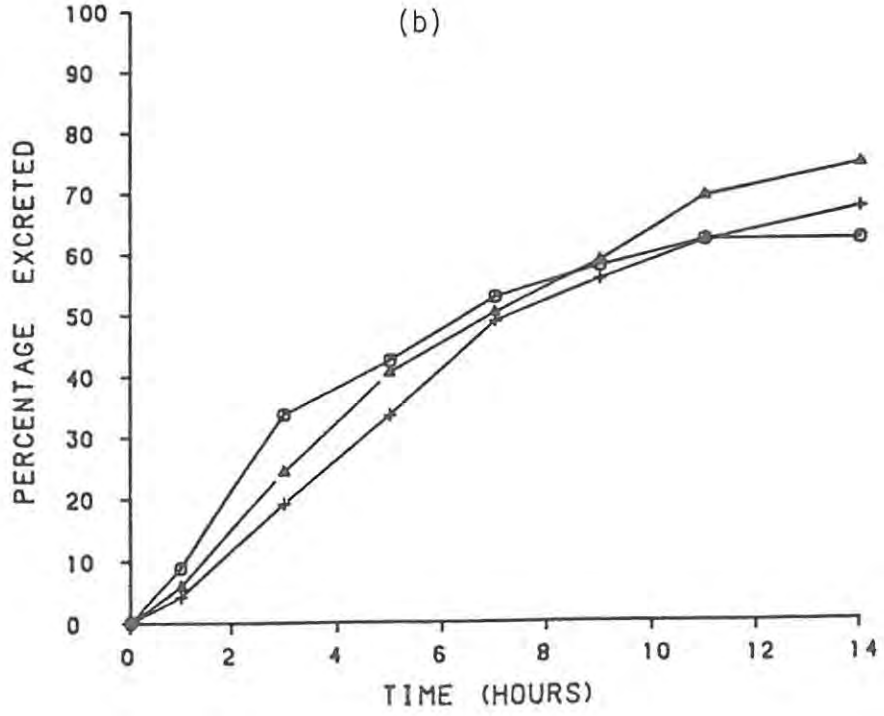
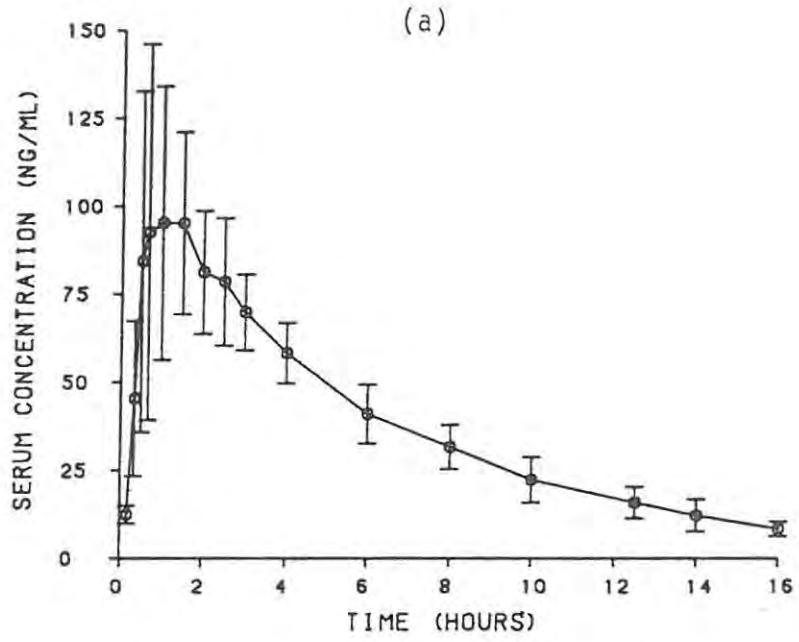


FIGURE 4.7 Mean serum concentrations (a) and urinary excretion profiles (b) ± SD of 5 subjects after the ingestion of 25 mg PPA.HCl solution.



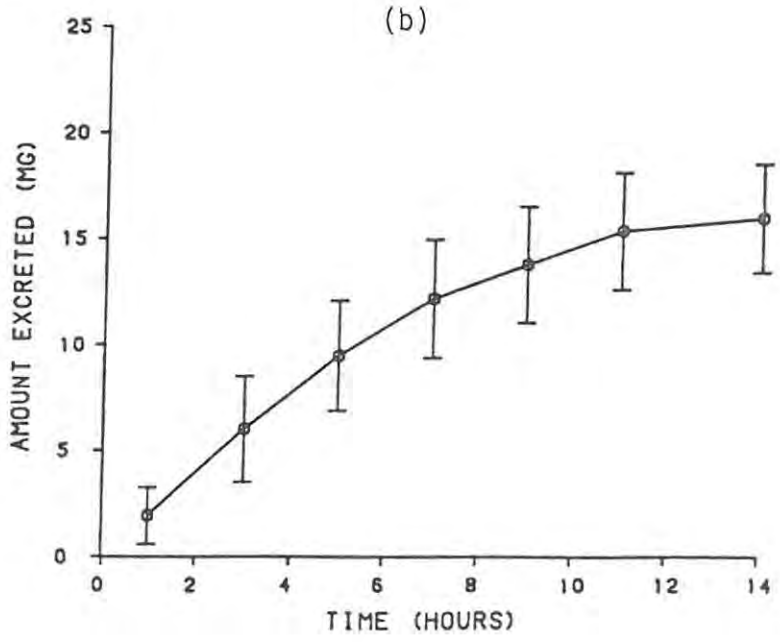
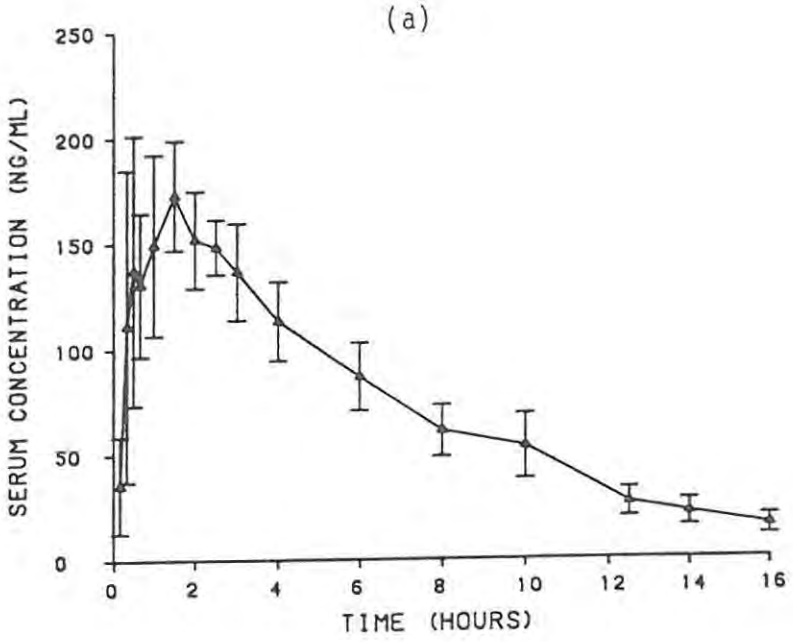


FIGURE 4.8 Mean serum concentrations (a) and urinary excretion profiles (b) ± SD of 5 subjects after the ingestion of 50 mg PPA.HCl solution.



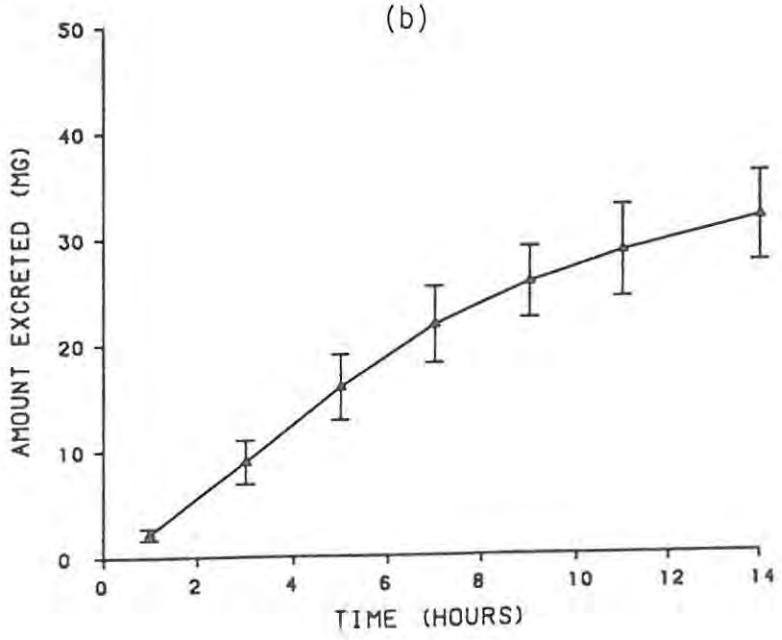
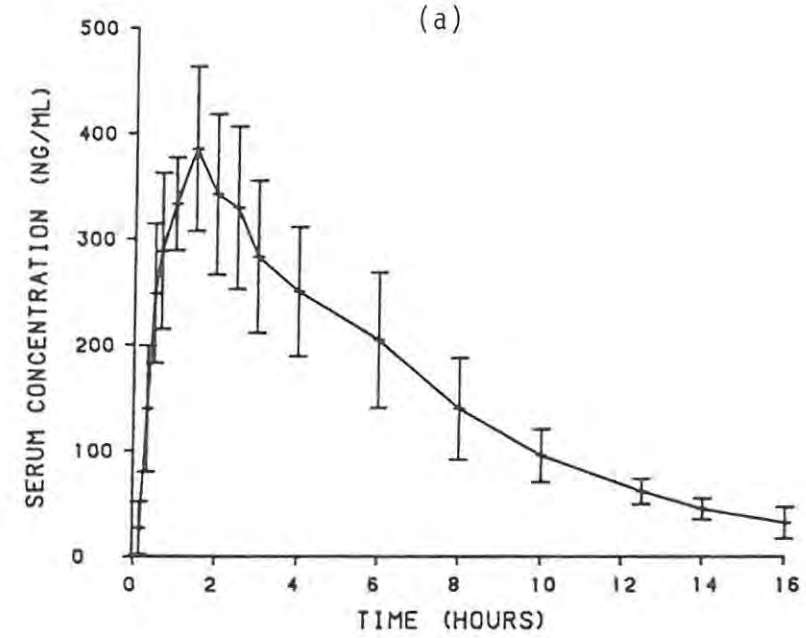


FIGURE 4.9 Mean serum concentrations (a) and urinary excretion profiles (b) ± SD of 5 subjects after the ingestion of 100 mg PPA.HCl solution.



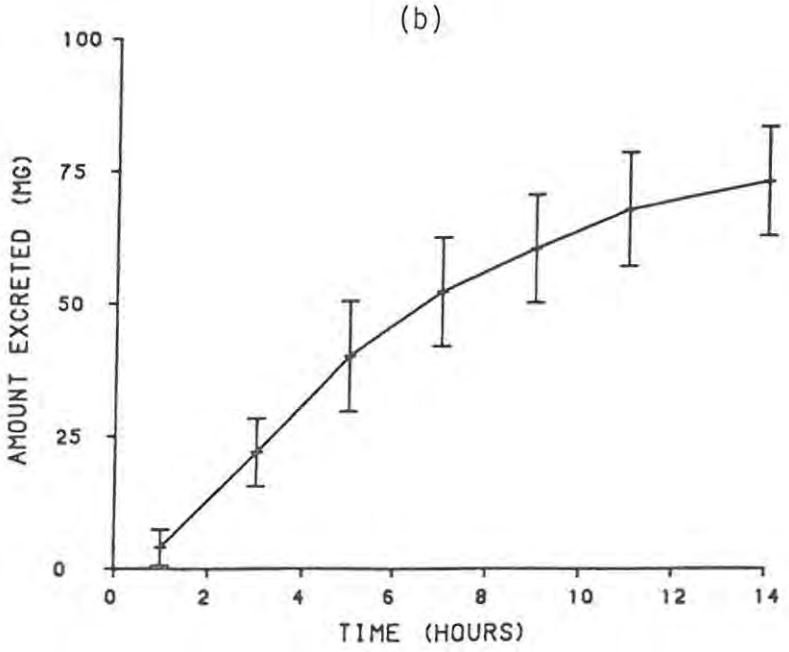
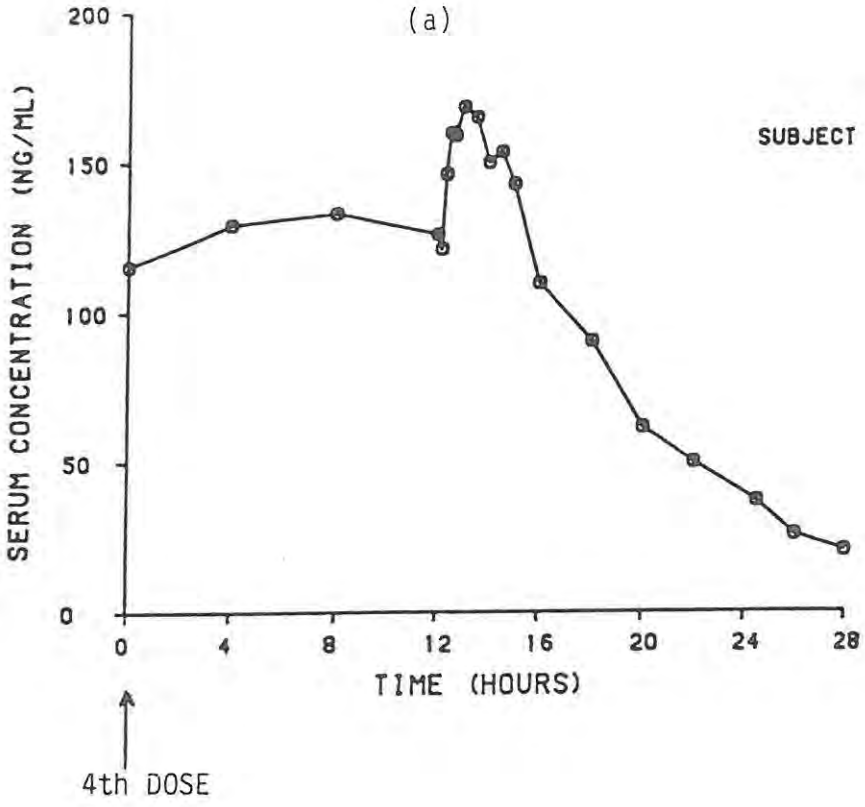


FIGURE 4.10 Serum concentrations (a) and urinary excretion profiles (b) from Subject GB after the ingestion of a 25 mg PPA.HCl solution every 4 hours for seven doses.



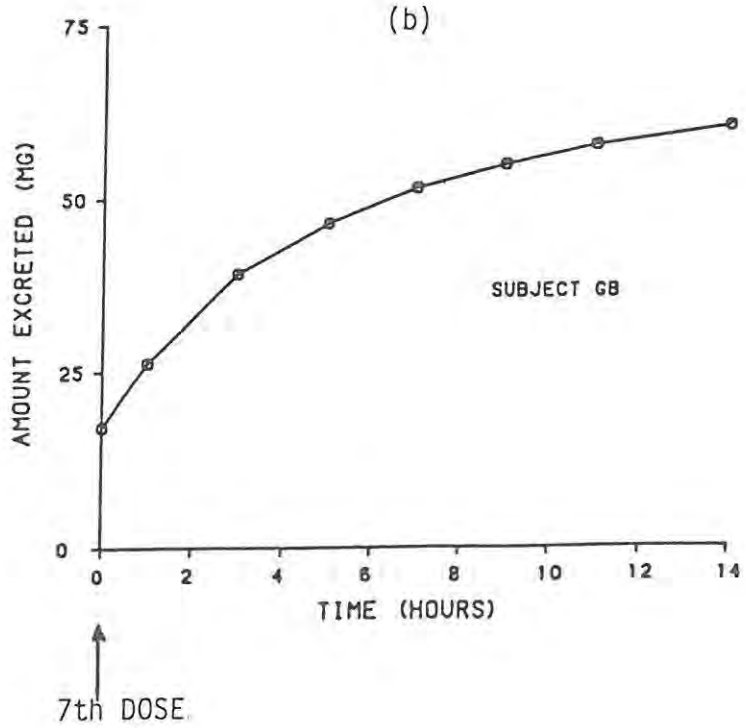
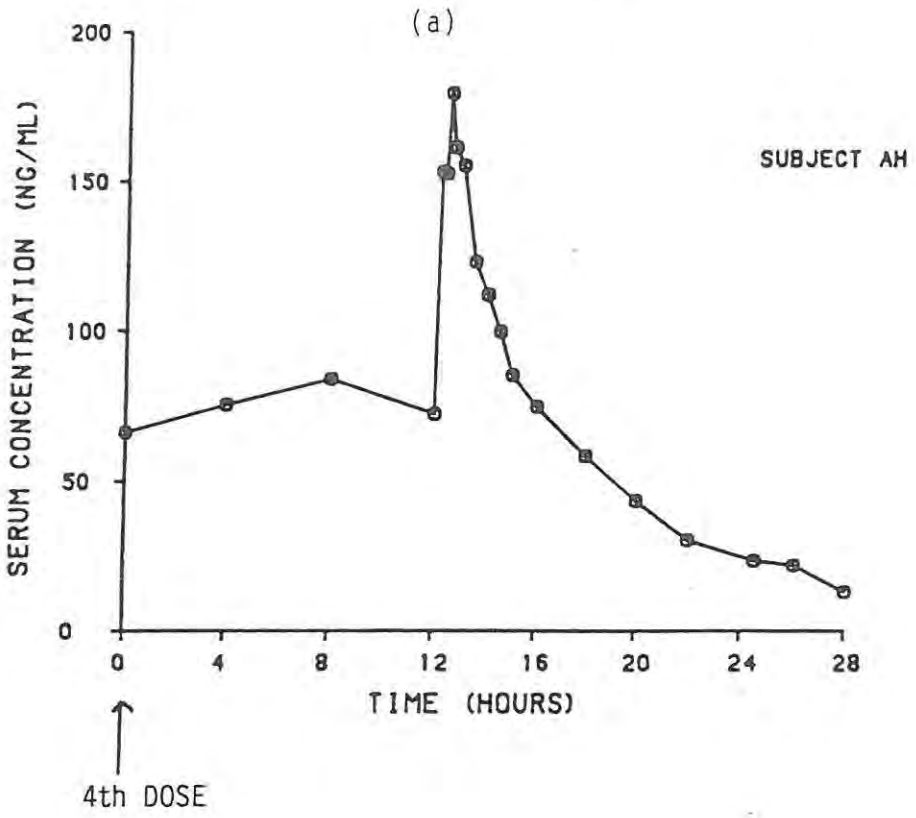


FIGURE 4.11 Serum concentrations (a) and urinary excretion profiles (b) from Subject AH after the ingestion of a 25 mg PPA.HCl solution every 4 hours for seven doses.



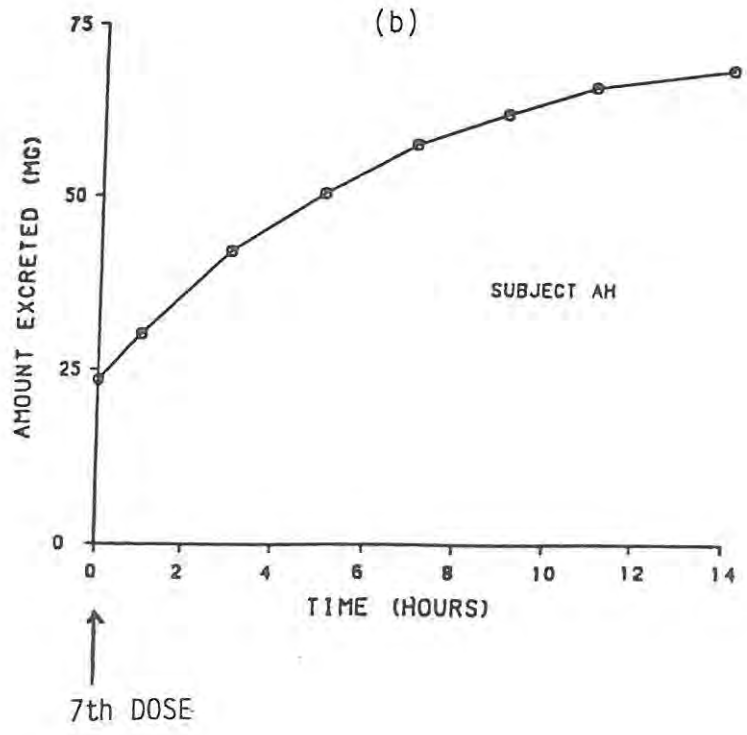
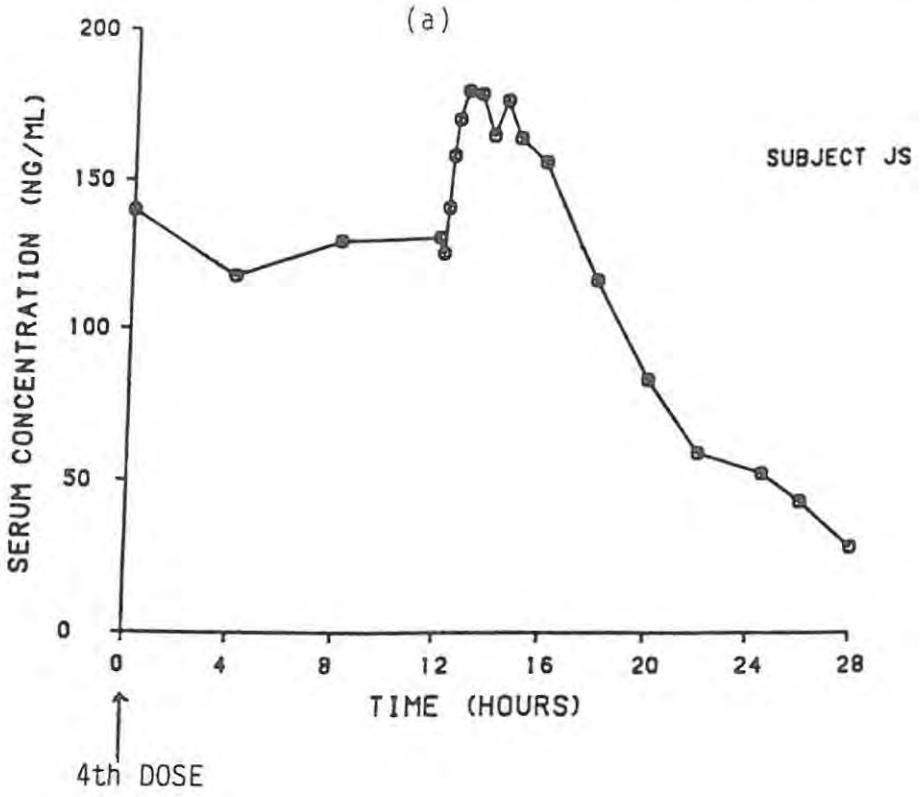


FIGURE 4.12 Serum concentrations (a) and urinary excretion profiles (b) from Subject JS after the ingestion of a 25 mg PPA.HCl solution every 4 hours for seven doses.



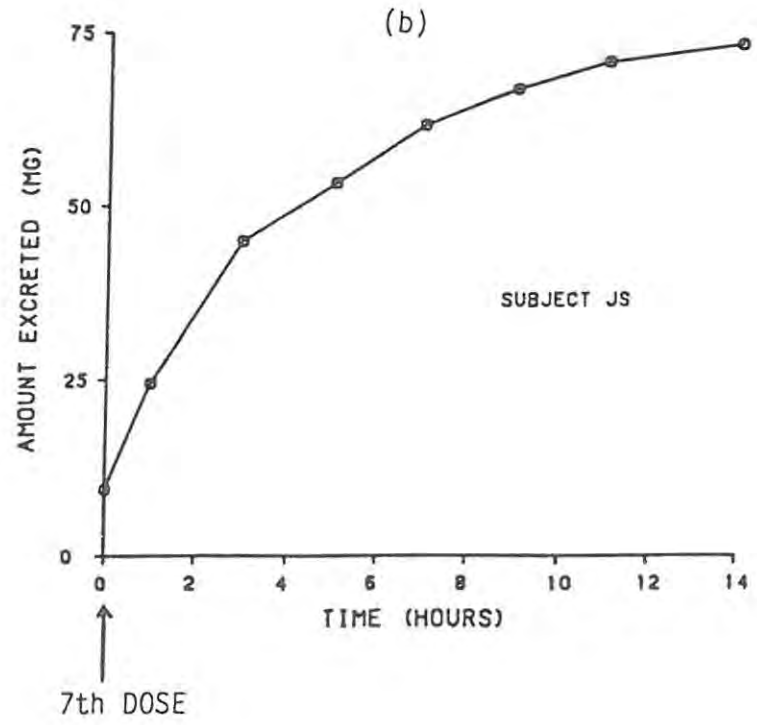
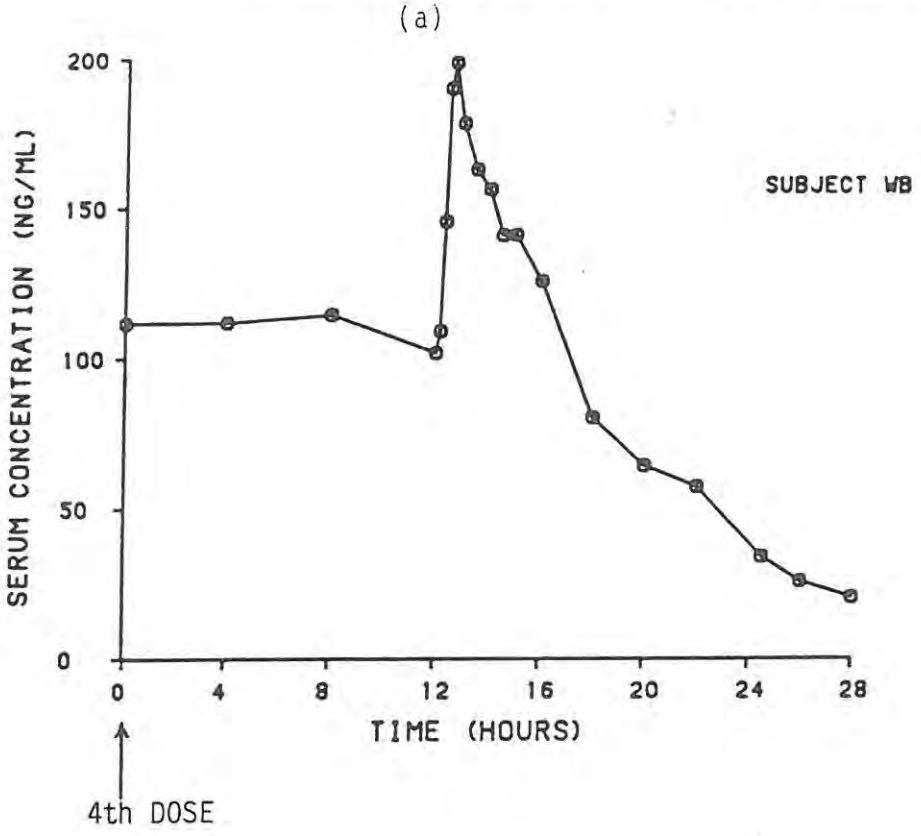


FIGURE 4.13 Serum concentrations (a) and urinary excretion profiles (b) from Subject WB after the ingestion of a 25 mg PPA.HCl solution every 4 hours for seven doses.



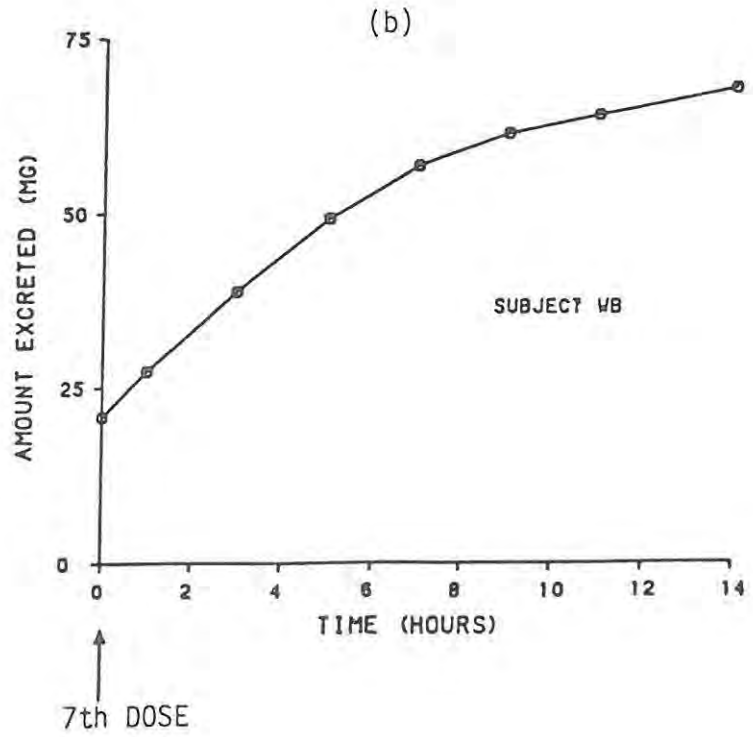
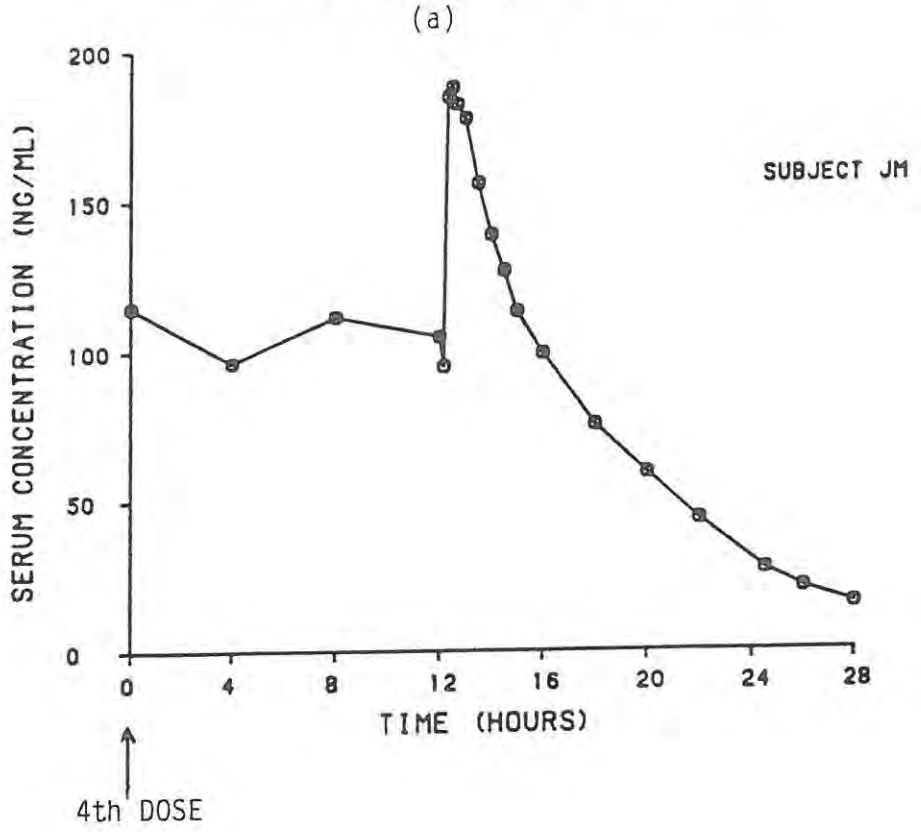
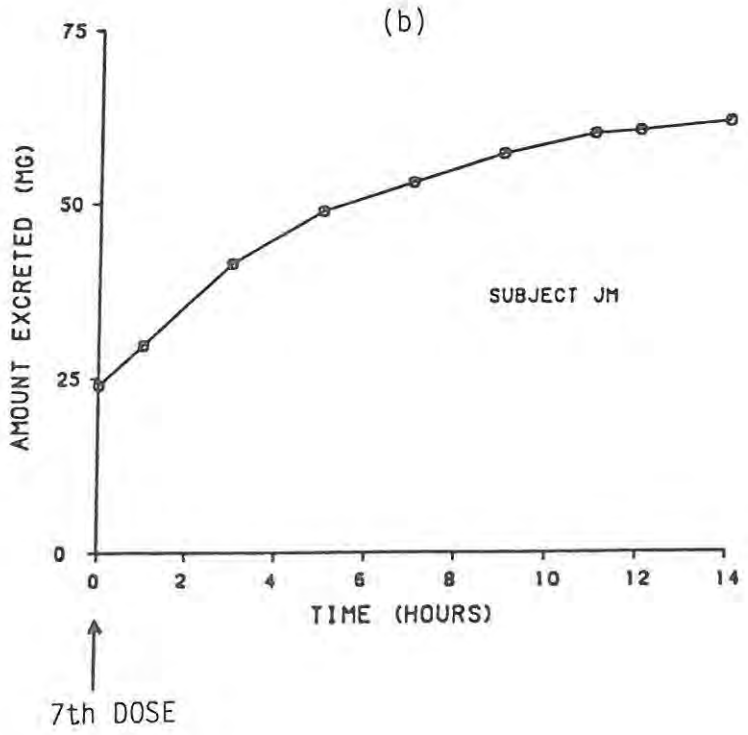


FIGURE 4.14 Serum concentrations (a) and urinary excretion profiles (b) from Subject JM after the ingestion of a 25 mg PPA.HCl solution every 4 hours for seven doses.





CHAPTER 5

PLASMA PROTEIN BINDING STUDY USING EQUILIBRIUM DIALYSIS

5.1 INTRODUCTION

It is commonly accepted that binding of drugs to plasma constituents plays an important role in the pharmacodynamics of highly bound substances. Plasma protein binding of drugs may cause detectable nonlinearity in log C versus t plots if doses are sufficiently high to approach saturation of binding sites and there is negligible elimination of the bound drug (249). Quantitative implications of protein binding in pharmacokinetics have been evaluated by means of numerical methods for one and two compartment models (250).

Although there are a variety of techniques for carrying out plasma protein binding measurements of drugs (251), equilibrium dialysis is one of the most popular, accurate and reliable methods available (251, 252). In this method, the serum sample and a non-protein drug-free buffer solution were placed on either side of a semi-permeable membrane. Unbound drug in plasma passes through the membrane until, at equilibrium, the concentration of drug in the buffer solution and that unbound in the plasma are equal. By measuring drug on both sides of the membrane, the fraction of drug in plasma unbound is readily assessed. A semi-permeable membrane permits diffusion of the small molecules (referred to as the dialysate or diffusate), and retains the macromolecules (the retentate). The diffusion of the small molecules occurs under the influence of a concentration gradient.

5.2 EXPERIMENTAL

5.2.1 The Dialysis Membrane

The dialysis membrane consisted of Visking cellulose dialysis tubing 4465-A2 (Union Carbide), with an average pore diameter of 480 nm. Besides cellulose, the tubing contains glycerine, water and small

amounts of sulphur compounds (approximately 0.1%). The latter may interfere with spectral methods of analysis. The membrane was therefore washed free of such materials and pre-treated by boiling in distilled water for 90 min. Excess treated membrane was stored in distilled water in a refrigerator (0-4°C) for further use.

The membrane is permeable to water and will allow low molecular weight compounds in aqueous solution to diffuse through while higher molecular weight materials such as proteins are retained.

5.2.2 Buffer Solution Preparation

The 0.05 M phosphate buffer used in these studies was prepared by adding 3.2 mL phosphoric acid to 1.0 litre of freshly prepared HPLC grade water. Sodium hydroxide pellets were added to the acid/water mixture with constant stirring on a magnetic stirrer until the desired pH of the buffer had been attained (pH 7.21).

5.2.3 The Dialysis Apparatus

Equilibrium dialysis was carried out in an apparatus consisting of two plexiglass blocks, each having five cavities and seven holes at the corners and sides. A small glass bead (3 mm diameter) was placed into each cavity to enable agitation. The pre-treated dialysis membrane was placed between the blocks with the cavities facing each other. The blocks were firmly held together by the use of seven stainless steel bolts and secured with stainless steel wingnuts. Each block has a threaded hole entering each cavity for the introduction of solution or removal of sample for analysis. The compartments on each side of the dialysis membrane measured 17.5 mm in diameter and 4.0 mm in depth. The volume of each compartment is approximately 1.0 mL. A serum sample (0.9 mL) was placed into the cavity on one side of the membrane, and the same volume of buffer was placed on the other side of the membrane. The entire assembly was then stoppered tightly by means of plastic screw-plugs. The cell was then attached to a shaking device which raised and lowered the cell 30 times/min at constant temperature for 24 hrs.

5.2.4 Serum Samples

Spiked serum samples were prepared as in Section 3.3.1.1 in concentrations ranging from 50 to 500 ng/mL. The *in vivo* serum samples were taken from previous clinical trials (see Section 4.2). Samples from subject AH, after the ingestion of 100 mg PPA.HCl in solution were used for concentrations from 95 to 280 ng/mL.

Prior to analysis the serum was thawed and allowed to reach room temperature. Ehrnebo *et al.* (253) showed that freezing and thawing of serum samples do not affect the binding capacity of serum.

5.2.5 Assay Procedure

After equilibrium, aliquots (0.750 mL) were removed from both sides of the membrane for analysis. Serum samples and corresponding buffer solutions were analyzed as described in Section 4.3.1. Each concentration was analyzed in duplicate. A calibration curve was constructed as in Section 3.3.1.4, from which the concentration of undialysed serum samples was obtained.

5.2.6 Calculation of Binding Capacity

Equation 5.1 (253) was used to determine the percentage of bound drug:

$$\% \text{ bound} = \frac{(C_{plasma} - C_{buffer}) \times 100}{C_{plasma}} \qquad (5\text{-}1)$$

where C_{plasma}=concentration of drug in the serum compartment after dialysis, and C_{buffer}=concentration of drug in the buffer compartment after dialysis.

5.3 RESULTS AND DISCUSSION

Plasma contains many proteins, albumin being the most abundant of all (see Table 5.1). Basic drugs, however, often associate more avidly with other proteins, particularly alpha$_1$-acid glycoprotein and lipoproteins, than with albumin (254). Proteins such as gammaglobulin, transcortin, fibrinogen and thyroid-binding globulin also bind drugs; they tend to be more specific with respect to the drugs they bind and usually have a much smaller capacity for binding.

TABLE 5.1 Major proteins to which drugs bind in serum.

Protein	Molecular weight	Normal range of concentrations (g/liter)	Normal range of concentrations (molar)
Albumin	65,000	35-50	5-7.4x10^{-4}
alpha$_1$-Acid glycoprotein	44,000	0.4-1.0	0.9-2.2x10^{-5}
Lipoproteins	200,000-3,400,000	Variable	

Table 5.2 lists hypothetical drugs and the changes expected in the volume of distribution, clearance and half-life on decreasing binding (increased fraction unbound). Drug-protein binding depends on four major factors: the affinity between drug and protein, the concentration of drug, the concentration of protein, and the presence of other substances which can either compete for the binding sites or, through allosteric effects, alter drug binding (251). Ingestion of food, particularly fats can reduce drug binding, although the mechanism is poorly understood (255). Table 5.3 lists the plasma binding values obtained for phenylpropanolamine in subject AH and spiked serum samples.

TABLE 5.2 Pharmacokinetic consequences of decreased binding to plasma proteins

Drug	Clearance	Volume of distribution (litres)	Volume of distribution	Clearance	Half-life
	[a]		[b]		
A	High	8	↔	↔	↔
B	High	100	↑	↔	↑
	[c]				
C	Low	8	↔	↑	↓
D	Low	100	↑	↑	↔

[a] Extraction ratio close to 1.0

[b] Symbols: ↔, little or no change; ↑, increase; ↓, decrease

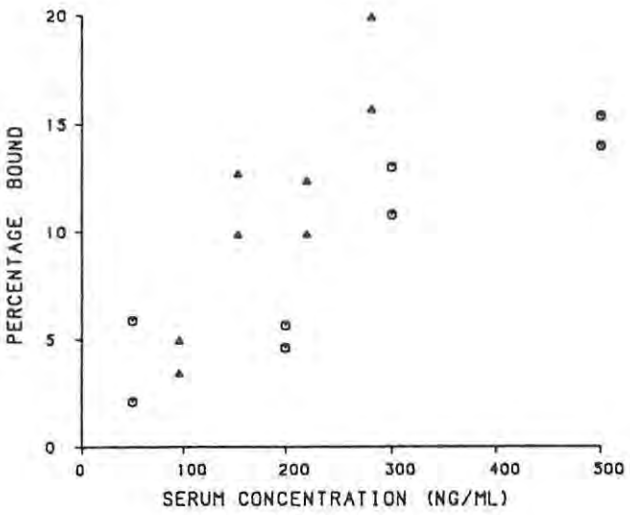
[c] Extraction ratio less than 0.1.

TABLE 5.3 Plasma Protein binding of Phenylpropanolamine.

Serum concentration (Total) ng/mL	Percentage Bound (%)		$\bar{X}$ (SD)	R.S.D.
	1	2		
95.76	4.91	3.40	4.155 (1.067)	25.69%
153.89	12.66	9.86	11.26 (1.97)	17.58%
220.03	9.87	12.32	11.09 (1.73)	15.60%
280.69	15.68	19.90	17.79 (2.98)	16.77%

Figure 5.1 illustrates a simple and useful graphical form of representation: it may be used to directly assess the fraction of bound drug and hence the bound concentration for a given total plasma concentration. Other methods most commonly used for graphically representing the results of a binding study in terms of linearization procedures include the Scatchard plot (256) or the double reciprocal plot (257).

FIGURE 5.1 Relationship between plasma binding of phenylpropanolamine and concentration of drug in human plasma.



The degree of binding of PPA in serum obtained from patient AH (Δ) varies with concentration. The binding in this volunteer is unaffected, however, by PPA administration itself: the control values (●), obtained from the spiked samples were no different to those of subject AH (Δ).

Any influence of plasma binding on drug elimination may best be understood by a consideration of clearance (258). Measured directly across an eliminating organ, clearance is the product of blood flow and extraction ratio. For drugs where the extraction ratio approaches unity, and clearance approaches blood flow, all drug passing through the organ is extracted, whether bound or not. For such drugs, clearance should be unaffected by changes in plasma binding. Only if the extraction ratio is low should clearance be affected by plasma binding (259).

The volume of distribution of most drugs far exceeds the plasma volume, so that only a small percent of the dose within the body is in plasma, and changes in plasma binding are too small to affect the unbound drug concentration. Changes in binding can affect the half-life ($t_{\frac{1}{2}}$), and when binding is diminished and urinary clearance is unaffected, the half-life of a poorly cleared drug is shortened (251).

Plasma protein binding of PPA was only slightly dependent on the concentration in serum. However, the objective here was to establish the influence of PPA serum concentration on the relative, not necessarily the absolute, drug binding capacity of plasma. Caution should be exercized in the interpretation of the results due to problems associated with protein binding studies using equilibrium dialysis. The problems include volume shift (260-262), adsorption of drugs to dialysis membranes and apparatus (263), pH shift as a result of bacterial growth (264) and effect of equilibration time on pH (265). No bacterial growth was observed in this study. However, a small shift in volume did occur. This volume shift was not accounted for as it was difficult to accurately quantify due to the small capacity (1 mL) of the dialysis cells.

CHAPTER 6

PHARMACOKINETIC ANALYSIS OF SOLUTION DATA

6.1. INTRODUCTION

The word "pharmacokinetics" means the application of kinetics to *pharmacon*, the Greek word for drugs and poisons. The purpose of pharmacokinetics is to study the time course of drug and metabolite concentrations and amounts in biological fluids, tissues and excreta, and to construct suitable mathematical models to interpret such data (266). A major problem in pharmacokinetics is to develop an adequate method to describe or interpret one or more data set(s), and frequently the method leads to a compartment model.

A "compartmentalized" system is an approximation for a biological system because variations in homogenicity of the media and diffusion processes are all interrelated with chemical changes. A compartment is thus an "average" rather than an exact state (267), and most pharmacokinetic models are deterministic in nature. The major contribution of a suitable model is that it allows the investigator to apply mathematical techniques, even though these compartments have no apparent physiologic or anatomic reality.

The behaviour of a drug in a biological system can usually be described in terms of either a one, two or multi-compartmental model, and most models are based on linear systems of differential equations. Kinetic linearity may be defined as direct proportionality of transfer rates to concentrations or concentration differences. It also implies that elimination of drug obeys first order kinetics. A consequence of a linear system in pharmacokinetics is that the total area under serum concentration-time curve is a linear function of the dose administered, if the fraction of drug which is protein-bound in plasma remains essentially constant in the range of concentrations of interest and if the fraction absorbed remains constant. Linear

kinetics assume that the tissues are far below the saturation level and that the concentration of drug in each tissue at a given time after dosing is a linear function of dose.

The three most common causes of nonlinear pharmacokinetics are: a) the operation of Michaelis-Menten or some other nonlinear elimination kinetics; b) the presence of saturable tissue-binding coupled with the fact that doses are administered which take one into the nonlinear region of binding; and c) the administration of doses which are high enough to take one into the nonlinear region of plasma protein binding.

6.2. LINEAR COMPARTMENT MODELS.

6.2.1. The One Compartment Model.

The one compartment model, the most simple pharmacokinetic model, depicts the body as a single homogenous unit. This model is useful for the pharmacokinetic analysis of serum concentration, and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation. To assume the body behaves as a one compartment model does not necessarily mean that the drug concentrations in all the body tissues at any given time are the same. However, a one compartment model does assume that any changes that occur in the plasma quantitatively reflect changes occurring in the tissue drug level.

A second criterion for using a one compartment model is that drug elimination occurs from the body in a first-order fashion. Drug elimination can occur from the body by many processes, including renal and biliary pathways, biotransformation, and excretion in the expired air. Glomerular filtration in the kidneys and passive diffusion into bile are simple first-order filtration processes, whereas tubular kidney secretion, biotransformation and biliary secretion frequently involve active processes. At low concentrations these latter processes can be approximated by linear kinetics with the apparent

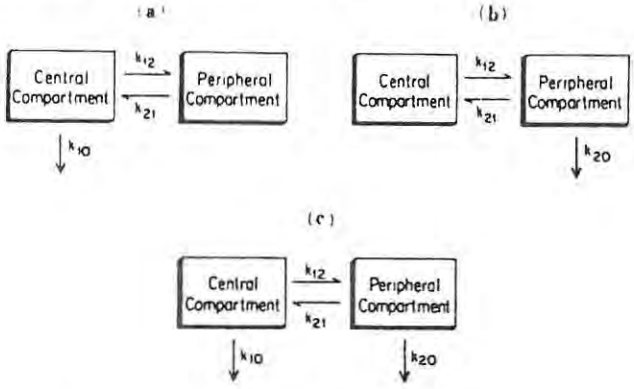
first-order elimination rate constant being the sum of the rate constants of a number of individual processes

6.2.2. The Two Compartment Model.

Most drugs entering the systemic circulation do not instantly distribute between the blood and other body fluids or tissues. Body fluids or tissues which are in equilibrium with the circulatory system form the central compartment which is accessible through blood sampling. The levels of drug associated with the central compartment should decline more rapidly during the distributive phase than during the post-distributive phase. In contrast, levels of drug in body fluids or tissues into which the drug distributes slowly (peripheral compartment) will increase to a maximum and then begin to decline during the distributive phase until eventually a steady state will be reached which terminates the distribution phase. The access of drugs to the various poorly perfused tissues may occur at different rates. Frequently however, for a given drug these rates appear to be very similar and cannot be differentiated based solely on plasma concentration-time data and consequently, all poorly perfused tissues are often "lumped" into a single peripheral compartment. The peripheral compartment of pharmacokinetic models are, at best, hybrids of several functional physiologic units. Drug elimination and transfer of drug between body compartments in multicompartment systems are assumed to occur by first-order processes.

There are 3 possible types of two compartment models (depicted in Fig. 6.1). They differ in that elimination occurs either a) from the central compartment, b) from the peripheral compartment, or c) from both these compartments. It is usual to assume that drug elimination from two-compartment systems takes place exclusively from the central compartment and all subsequent equations in this section are based on this assumption. The basis of this assumption is that the major sites of biotransformation and excretion i.e., the liver and kidneys, are well perfused with blood and are therefore rapidly accessible to drug in the systemic circulation.

FIGURE 6.1 Schematic representation of three types of two compartment systems consisting of a central compartment and a peripheral compartment.



Definition of terms:

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

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

k_{10} = first order rate constant for elimination of drug by all processes from central compartment.

k_{20} = first order rate constant for elimination of drug by all processes from peripheral compartment.

6.3 ASSESSMENT OF PHARMOKINETIC PARAMETERS

6.3.1 Bioavailability

Bioavailability has been defined as the measurement of both the relative amount of an administered dose that reaches the general circulation (i.e. the extent of absorption of a given dose) and the rate at which this occurs (268). In all cases, except where a drug is administered intravenously in the form of a true solution, the drug has to be released from the dosage form and then be absorbed into systemic circulation by passing through various membranes. Differences in onset, intensity and duration of the pharmacological or clinical

effect are primarily due to differences either with the patient (physiologically modified bioavailability) or the dosage form (dosage form modified bioavailability).

In testing bioavailability one has to carefully design the protocol in order to minimize physiologically based modifications of bioavailability where possible. These include age, sex, physical state of the patient, time of administration, stomach emptying rate, type and amount of food and body weight, and the logical consequence is a true cross-over design.

The *in vivo* bioavailability of a drug product is demonstrated by both the rate and extent of absorption of the active ingredient. There are several possible approaches to measure bioavailability, namely blood level data, urinary excretion data and pharmacologic data. If blood level studies are not feasible, they can sometimes be substituted by urinary excretion studies. If the drug cannot be accurately assayed in biological fluids, a pharmacologic method can be used to substitute for blood level or urinary excretion studies.

Although, in general, we assume that the drug concentration in blood, plasma or serum correlates with the pharmacologic response, it is not applicable to all drugs and the actual bioavailability testing does not attempt to determine the drug concentration at the site of the drug action but in the systemic circulation. Even if the biophase, the locus of interaction between the drug and the cell, is not in the systemic circulation but somewhere in the tissues, the drug concentration in the systemic circulation may still correlate with the therapeutic response, since the transfer of free, non-protein bound drug from the circulation to the tissue depends on the concentration gradient and can be described mathematically. Exceptions are where active transport is involved.

The most precise evaluation of extent and rate of bioavailability is obtained from single or multiple intravenous dose blood level studies followed by single- or multiple dose urinary excretion studies. The 3 parameters that characterize the rate an extent of bioavailability are a) area under curve (AUC) b) actual peak height and c) time to reach the peak. When a given dose of drug is administered at uniform time intervals, the serum concentration-time profile in each dose interval will change in such a manner that the maximum and minimum concentration both increase with increase in number of doses until eventually a steady state is reached. Linear pharmacokinetic theory indicates that the area from zero to infinity under a single dose curve will be equal to the area within a dosage interval at the steady state, provided that the dose, the fraction of the dose which is absorbed, the dosing interval and the mean clearance remain constant in a given subject.

Pharmacokinetic methods for determining availability are often model-independent and when dose independent kinetics are assumed, all distribution and elimination processes are assumed to proceed in a first-order fashion. The availability of a drug or its active metabolite(s) to the end-organ is controlled by three factors: a) the rate and extent of release of drug from the dosage form b) the so-called "first pass" effect whereby, when a drug is administered orally, only a certain fraction presented to the gastrointestinal wall and the liver reaches the general circulation intact, and c) the combined processes of plasma protein-binding, tissue-binding, drug distribution, metabolism and urinary, fecal or lung excretion.

6.3.2. Assessment of Extent of Absorption

Absorption of drugs involves the process of uptake of the compound from the site of administration into the systemic circulation (269). Methods of assessing extent of absorption which do not involve the determination of the absorption rate, rely on fewer assumptions and are easier to apply to experimental data than methods for assessing the absorption rate. Calculation of the fraction, F, and the dose, D, which is absorbed and reaches the general circulation reflects the extent of absorption.

The most frequently used approach to the measurement of extent of absorption is to compare the area under the concentration-time curve, and the method for estimating area under the curve (AUC) is usually the linear trapezoidal method:

$$AUC = \left[\frac{C_n + C_{n-1}}{2}\right](t_n - t_{n-1}) \qquad (6\text{-}1)$$

Errors arise if sampling intervals are long relative to the half-life of the drug and if the function to be assessed shows marked curvature. The use of the linear trapezoidal method results in considerable under-estimation of the area during the absorption phase and over-estimation of the area in the post-absorption phase, where the curve decreases in an approximately exponential manner. Chiou (270) and Yeh and Kwan (271) investigated errors associated with trapezoidal integration.

The log trapezoidal method, which employs an exponential rather than a linear approximation to the data, has been applied to the post-absorption phase where the function declines monoexponentially. The log trapezoidal equation is shown below:

$$AUC = \frac{C_n - C_{n-1}}{(1/t_n - t_{n-1}) \ln (C_n/C_{n-1})} \qquad (6\text{-}2)$$

To determine AUC the assumption is made that concentration decreases monoexponentially after the last data point. When elimination is by a first order process, this decline must be measured for a sufficient time prior to the last data point to enable an accurate estimation of

the slope of the terminal portion of a semilogarithmic plot of concentration versus time. Another model-independent method proposed for assessing the extent of absorption is based on estimating the renal clearance, plasma clearance and urinary excretion of the unchanged drug (272).

6.3.3. Assessment of Rate of Absorption

Drug absorption after oral administration is frequently assumed to occur by an apparent first-order process, and is therefore quantified in terms of an apparent first-order absorption rate constant ka. This may be accomplished by appropriate evaluation of serum concentration-time data and sometimes urinary excretion data. Numerous methods for determining absorption rates have been reviewed (273-277). The maximum blood concentration, C_{max}, and the time at which the maximum occurs, t_{max}, give a crude indication of the absorption rate and are estimated directly from the serum concentration versus time data. Although the rate of absorption controls t_{max}, absorption which continues after t_{max} has no influence on this parameter.

In the statistical moment theory, absorption is regarded as a stochastic process. Since statistical moments are characteristic of the shape of the statistical distribution curve, such as the time course of serum concentration following a single dose of the drug, they are only dependent on the observed time course data and are model-independent. This approach allows separation of the absorption from the disposition phase and summarizes in a single figure the salient features of the absorption rate. The zero moment represents the AUC which has been discussed. The area under the first moment of the curve (AUMC) is defined as the product of the area under the curve of time, t, from zero time to infinity. Using this approach the mean residence time (MRT) can be calculated as follows:

$$MRT = \frac{\int_0^\infty t C_p \, dt}{\int_0^\infty C_p dt} = \frac{AUMC_\infty}{AUC_\infty} \qquad (6\text{-}3)$$

The MRT can be defined as the mean time for intact drug molecules to transit theoughout the body and it yields information on all kinetic processes including *in vivo* release from the dosage form, absorption into the body and all disposition processes.

Mass balance methods are based on the assumption of a compartmental model and amounts absorbed at any time are calculated from the sum of the amounts in each compartment plus that which has been eliminated. The Wagner-Nelson method (278) is based on the one compartment open model. The Loo-Riegelman method (279) is based on the two compartment open model and requires that the drug be administered intravenously. In application of both methods, the amount of drug absorbed to some time divided by the volume of distribution is obtained as a function of time. The main application of the Wagner-Nelson method would be in studying absorption profiles of drugs in which compartment kinetics have been shown to exist. Assuming first order elimination, the fraction absorbed (Fa) to time, t, is equal to:

$$F_a = \frac{C_p + k\ AUC_0^t}{k\ AUC_0^\infty} \qquad (6\text{-}4)$$

The rate constant, k, may be estimated from the slope of the terminal concentration-time data if absorption is sufficiently rapid.

Other approaches to the assessment of absorption rates include deconvolution methods, which include a) finite differences methods (280-282)), b) the least squares method (283), c) exact methods (284, 285), d) curve fitting methods (286), e) nonparametric methods (287), and f) first order rate constant methods (288).

6.4 RESULTS AND DISCUSSION

6.4.1 Bioavailability Parameters

The 25 mg, 50 mg and 100 mg solution data were initially treated using model-independent methods. Maximum PPA concentrations, C_{max}, and the time to reach this concentration, t_{max}, were determined from individual PPA serum concentration profiles. The terminal rate constant, K_{el}, was calculated from the terminal slope of the semilog plot of serum concentration versus time. From these data, the elimination half-life, $t_{\frac{1}{2}}$, was calculated where

$$t_{\frac{1}{2}} = \frac{0.693}{K_{el}} \qquad (6\text{-}5)$$

The apparent volume of distribution, V, was estimated using the following equation, assuming F=1:

$$V = \frac{F.D}{AUC_{\infty}.K_{el}} \qquad (6\text{-}6)$$

where D is the dose in µg.

The above equation should include a bioavailability term, F. In the absence of intravenous data absolute bioavailability cannot be determined. However, a fairly accurate estimate of bioavailability may be obtained from the portion of dose recovered in the urine at time infinity. One investigator reported that 90% of the 25 mg oral dose was excreted in the urine as unchanged PPA (241, 246), while in another report (247) 80-90 % of the dose was excreted unchanged within 24 hours.

From our studies, recovery of PPA from the urine after the ingestion of the PPA solutions was monitored for 14 hours with 54% to 81% of the unchanged drug (free base) being recovered. Mean urinary recoveries obtained from the 25, 50 and 100 mg solution studies were 64.1 %, 63.3 % and 72.9 % respectively. Recovery could have been greater if urine had been collected for a longer time as PPA was still detectable in the blood after 24 hours (Pilot trial).

Area under the curve (AUC) data was calculated using the linear trapezoidal equation in the absorption phase and the log trapezoidal method in the post-absorption phase where the function declines monoexponentially.

TABLE 6.1

BIOAVAILABILITY PARAMETERS FOR THE 25, 50 AND 100mg SOLUTION DATA

TEST DOSE	SUBJECT	PARAMETERS					
		t_{max} (hrs)	C_{max} (ng/ml)	AUC_{∞} (ng/ml.hr)	$Ke\ell$ (hr)	$t_{½}$ (hrs)	V (litre/kg
25 mg PPA.HCℓ SOLUTION	GB	3	69.39	536.7	0.176	3.93	4.3
	AH	0.67	81.81	491.1	0.162	4.28	2.7
	JS	1.5	102.01	758.9	0.144	4.81	3.6
	WB	1.5	127.16	719.0	0.168	4.12	2.4
	JM	0.67	185.25	690.4	0.144	4.79	3.3
	MEAN	1.47	113.12	639.2	0.159	4.39	3.3
	SD	0.953	45.86	118.1	0.014	0.39	0.75
50 mg PPA.HCℓ SOLUTION	GB	1.0	149.09	1116.3	0.185	3.75	4.1
	AH	0.5	204.44	1034.3	0.194	3.58	3.0
	JS	1.5	193.60	1432.0	0.154	4.51	3.7
	WB	1.5	157.21	1277.9	0.175	3.95	2.7
	JM	0.5	207.06	1392.2	0.198	3.50	2.4
	MEAN	1	182.28	1250.6	0.181	3.86	3.2
	SD	0.5	27.22	172.15	0.018	0.40	0.70
100 mg PPA.HCℓ SOLUTION	GB	1.5	480.60	2679.2	0.165	4.19	3.8
	AH	1.0	308.43	1920.7	0.160	4.32	3.8
	JS	2.0	437.13	3552.8	0.186	3.73	2.5
	WB	2.5	343.80	2408.8	0.291	2.39	1.8
	JM	1.5	414.16	2913.3	0.159	4.39	2.9
	MEAN	1.7	396.82	2694.4	0.168	3.80	3.0
	SD	0.57	69.94	605.2	0.013	0.83	0.86

TABLE 6.2 BIOAVAILABILITY PARAMETERS FOR MULTIPLE DOSE STUDY

	PARAMETERS						
SUBJECT	T_{max} (hr)	C_{max} (ng/mL)	AUC (ng/mL.hr^{-1})	K_{el} (hr^{-1})	$t_{½}$ (hrs)	$\bar{C}$ (ng/mL)	C_{min} (ng/mL)
GB	1.0	168.63	587.8	0.15	4.62	117.15	125.96
AH	0.5	179.53	461.1	0.14	4.95	109.15	72.53
JS	1.0	179.67	660.2	0.134	5.17	172.08	130.79
WB	0.67	198.67	704.9	0.15	4.62	159.15	101.73
JM	0.5	187.47	550.4	0.166	4.17	131.88	104.65
Mean	0.734	182.79	572.9	0.148	4.71	137.88	107.13
± SD	0.25	11.12	73.9	0.012	0.37	26.98	23.16

TABLE 6.3 Fluctuation Index (FI) and Percentage Fluctuation values for the multiple dose study.

Subject	F.I.	Percentage Fluctuation
GB	0.36	33.87%
AH	0.98	147.52%
JS	0.28	37.37%
WB	0.61	95.29%
JM	0.63	79.14%
MEAN	0.57	77.83%
±SD	0.27	47.62

It is sometimes desirable to determine availability of a drug based on steady state plasma drug levels following multiple dosing (289). The drug concentration in the serum at any given point in time during a dosing interval will increase as the number of doses increases and approach a constant level. After multiple dosing for a time equal to approximately four times the biologic half-life of a drug, the serum concentration is within 10% of its plateau or steady-state level. After a period of time equal to 7 half-lives, the drug concentration, at any point in time during a dosing interval is within 1% of the plateau level (289).

The parameters t_{max}, $t_{1/2}$, AUC, V and the terminal rate constant K_{el} were obtained from the individual serum concentration versus time data at steady state. Other parameters obtained from serum concentration-time data were the maximum and minimum steady state serum concentrations, Cmax and Cmin. A parameter which is very useful in multiple dosing is the average concentration of drug in plasma at steady state, $\bar{C}$. This parameter can be predicted by employing

$$\bar{C} = \frac{AUC}{\tau} \tag{6-7}$$

where τ is the dosage interval and AUC is the area within the dosage interval at steady state using the linear trapezoidal equation. This equation does assume, however, that V and K_{el} are constant over the entire dosing period. From simple geometric considerations, $\bar{C}$ represents some plasma concentration between Cmax and Cmin. A limitation of the $\bar{C}$ approach is that it gives no information about the fluctuations in plasma levels. Thus, the fluctuation index, (FI), and percentage fluctuation were calculated where:

$$FI = \frac{Cmax - Cmin}{\bar{C}} \tag{6-8}$$

$$\text{and Percentage Fluctuation} = 100 \cdot \frac{Cmax - Cmin}{Cmin} \tag{6-9}$$

The administration of a drug on a multiple dose regimen will result in the accumulation of drug in the body. By knowing the elimination rate constant, the extent to which a drug would accumulate in the body following a fixed dosing regimen can be calculated by employing

$$R = \frac{1}{1 - e^{-K_{el}\tau}} \tag{6-10}$$

where R is the index of accumulation, which is independent of dose.

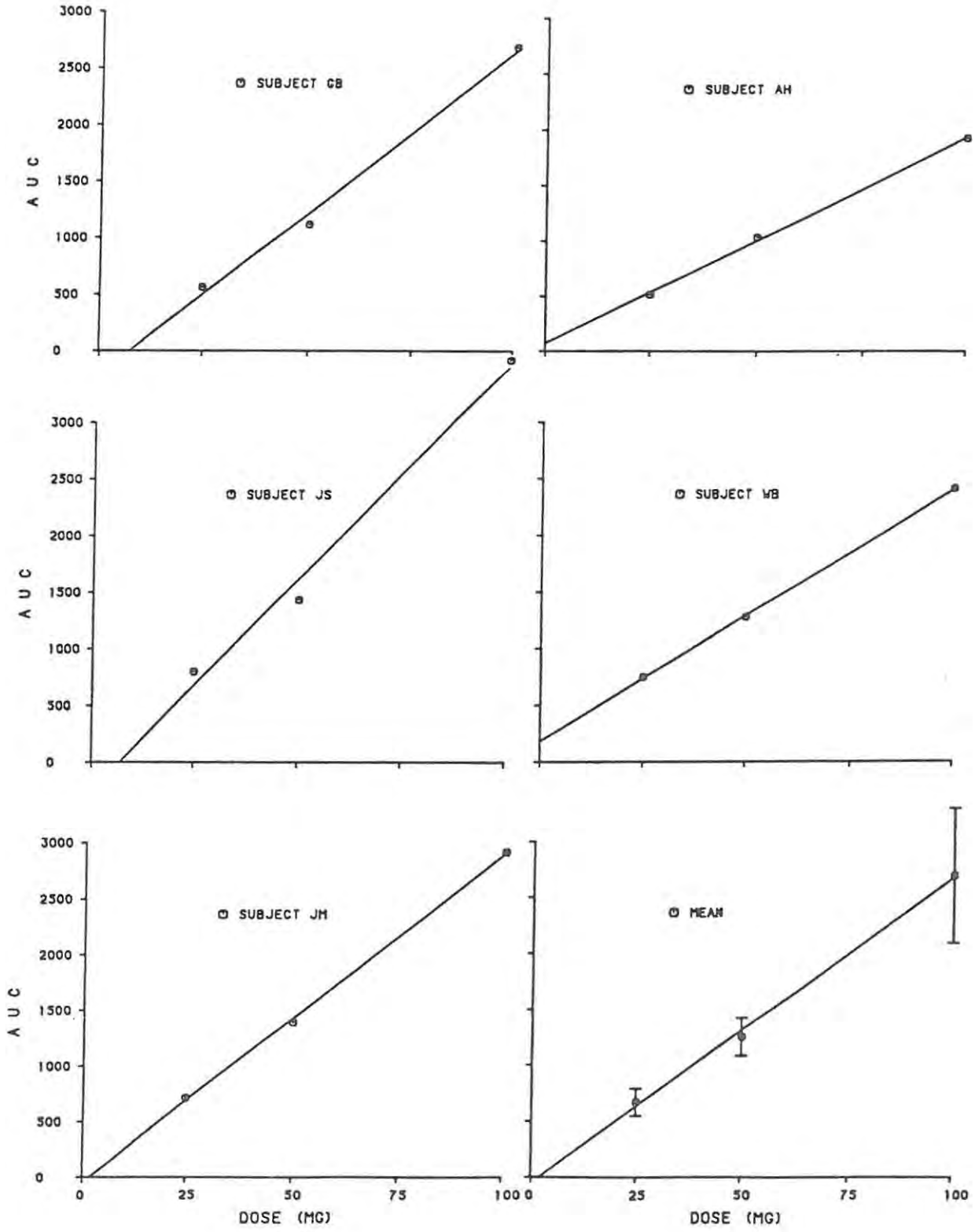
In most subjects , the t_{max} tended to increase with increase in dose. Subjects AH and JM displayed the shortest t_{max} values in both the multiple and single dose trials. The apparent volume of distribution is large, which would account for the low concentrations of PPA in the serum. This rather high distribution volume indicated that PPA is extensively bound to extravascular sites, which agrees with results of a previous study (290).

The half-life values from the single dose study for all subjects did not differ to any great extent, except for subject WB, who displayed a larger K_{el} and consequently a shorter half-life than the other subjects in the 100 mg solution study. There was a general trend for the elimination rate constant to decrease in the multiple dose study, and consequently the half-life increased, ranging from 4.2 to 5.2 hours. The AUC_{∞} values for subject JS was the highest of the 5 subjects, partly due to the slow elimination of the drug.

An important consequence of a linear system in pharmacokinetics is that the total area under the blood or serum concentration-time curve, is a linear function of the dose administered. This is one of the best tests of linearity in pharmacokinetics. The area under the curve values for each subject was plotted versus the respective dose administered (Fig. 6.2). A straight line fit of the data was made by least-squares linear regression analysis, and for all subjects the AUC_{∞} values increased proportionately with an increase in dose. Phenylpropanolamine thus appears to exhibit linear kinetics as can be seen particularly from results exhibited by subjects JM, AH and WB.

There was an approximately 10% difference in bioavailability based on AUC between the single- and multiple-dosing trials with the same PPA dose (25 mg) given to the same subject. It was therefore very likely that there was a slight change in the clearance between the single- and multiple-dosing tests. A small change in the elimination rate constant is evident when the single dose (25 mg) is compared with the multiple dose profiles. The elimination rate constants for the multiple dose tended to fluctuate about a mean value of 0.148 hr^{-1},

FIGURE 6.2 Area under the curve (AUC) values for each subject versus the respective dose administered.



which is smaller than the value estimated after the single dose. The reason for this apparent change in half-life remains obscure. Whether it is due to a real change in the pharmacokinetics of the drug upon repetitive dosing is not known. Data collected during steady-state conditions contain little information about the volume of distribution parameter, although the pharmacokinetic parameters estimated from the single dose studies are in good agreement with previously reported parameters Dowse *et al.* (290).

Of all the subjects, JS had the highest AUC values for both the single and multiple dose studies. The elimination rate constant for all subjects in the single dose trials did not differ to any great extent except for subject WB who displayed a larger K_{el} and consequently a shorter half-life than the other subjects in the 100 mg trial. The AUC_{∞} value for subject AH was the lowest of the 5 subjects. Subject AH was large in stature and had the highest body weight, thereby possibly accounting for this phenomenon. Serum trough levels after 7 oral doses of PPA.HCl every 4 hours are given in Table 6.4, and averaged 109.3 $\pm$ 21.7 ng/mL.

TABLE 6.4 Serum trough levels of the multiple dose study.

Subject	Serum concentration (ng/mL)				Mean (SD)
GB	115.3	129.3	133.1	125.9	125.9 (7.65)
AH	66.3	75.5	84.1	72.5	74.6 (7.40)
JS	139.9	117.3	129.6	130.8	129.4 (9.28)
WB	111.5	111.7	114.2	101.7	109.7 (5.52)
JM	114.9	96.2	111.5	104.6	106.8 (8.26)
					109.3 (21.7)

6.4.2 Wagner-Nelson Absorption Plots

The solution data were treated assuming a linear one compartment model with first order absorption. The Wagner-Nelson equation was applied to the serum concentration-time data to yield absorption plots of fraction absorbed versus time. Wagner-Nelson plots of serum concentration-time curves are depicted in Figs. 6.3-6.5. The absorption rate constants were calculated from the slope of the semilog plot of fraction of dose remaining to be absorbed versus time and are shown in Table 6.5.

TABLE 6.5 Values of ka for the 25, 50 and 100 mg solution studies.

TEST DOSE	SUBJECTS					MEAN (SD)
	GB	AH	JS	WB	JM	
25mg	1.16	4.16	2.24	1.85	7.49	3.38 (2.55)
50mg	1.69	4.63	1.54	1.63	9.69	3.83 (3.52)
100mg	1.76	4.45	1.15	1.17	7.83	2.20 (1.37)

Subjects AH and JM exhibited a much larger absorption rate than the other subjects, which is consistent with the short t_{max} from these subjects. Absorption of the drug is rapid, with peak concentrations occurring between 40 min and 3 hours after ingestion, which agrees with the results of previous studies (290).

FIGURE 6.3 Wagner-Nelson absorption plots of serum concentration-time curves after administering the 25 mg PPA.HCl solution.

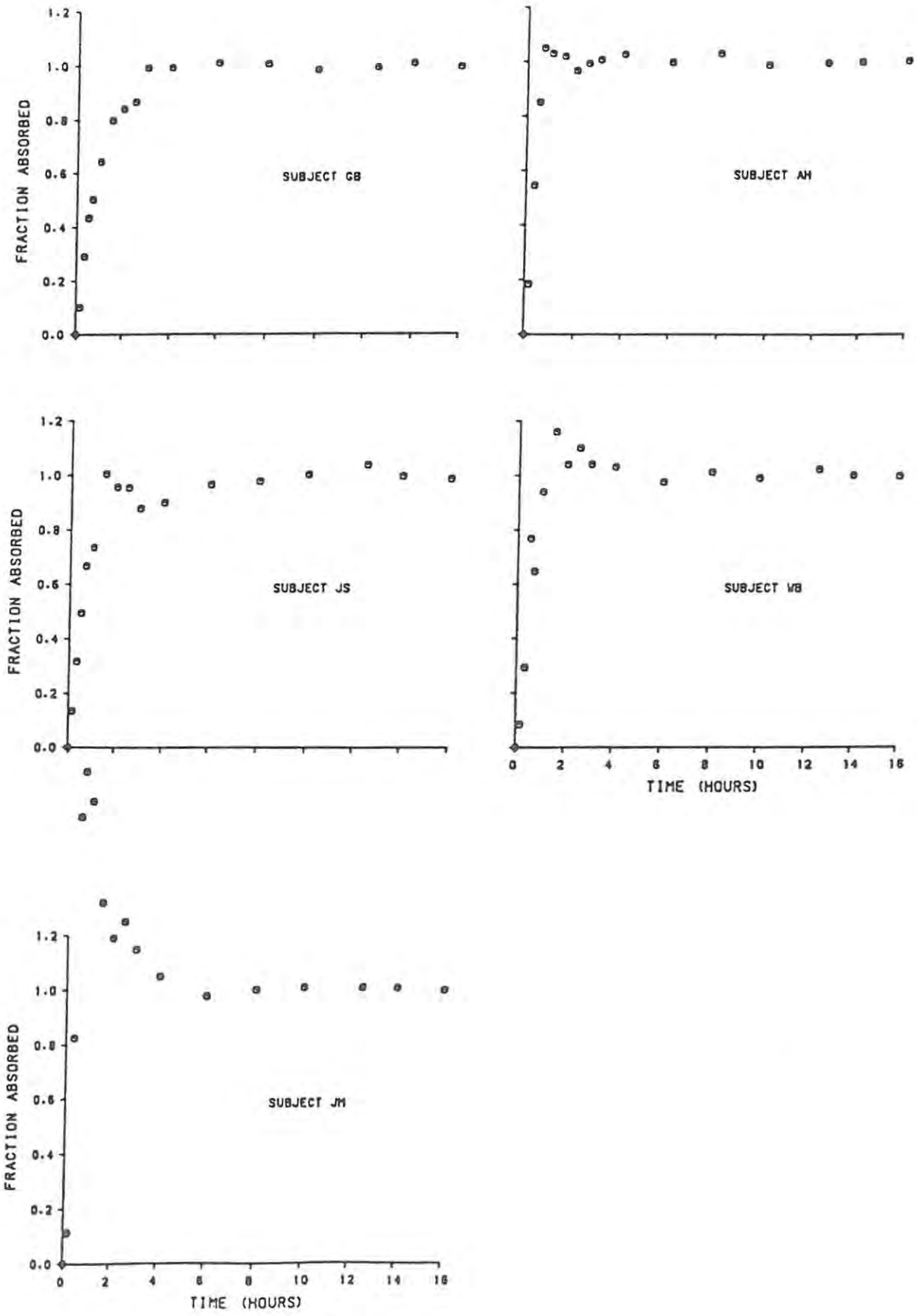


FIGURE 6.4 Wagner-Nelson absorption plots of serum concentration-time curves after administering the 50 mg PPA.HCl solution.

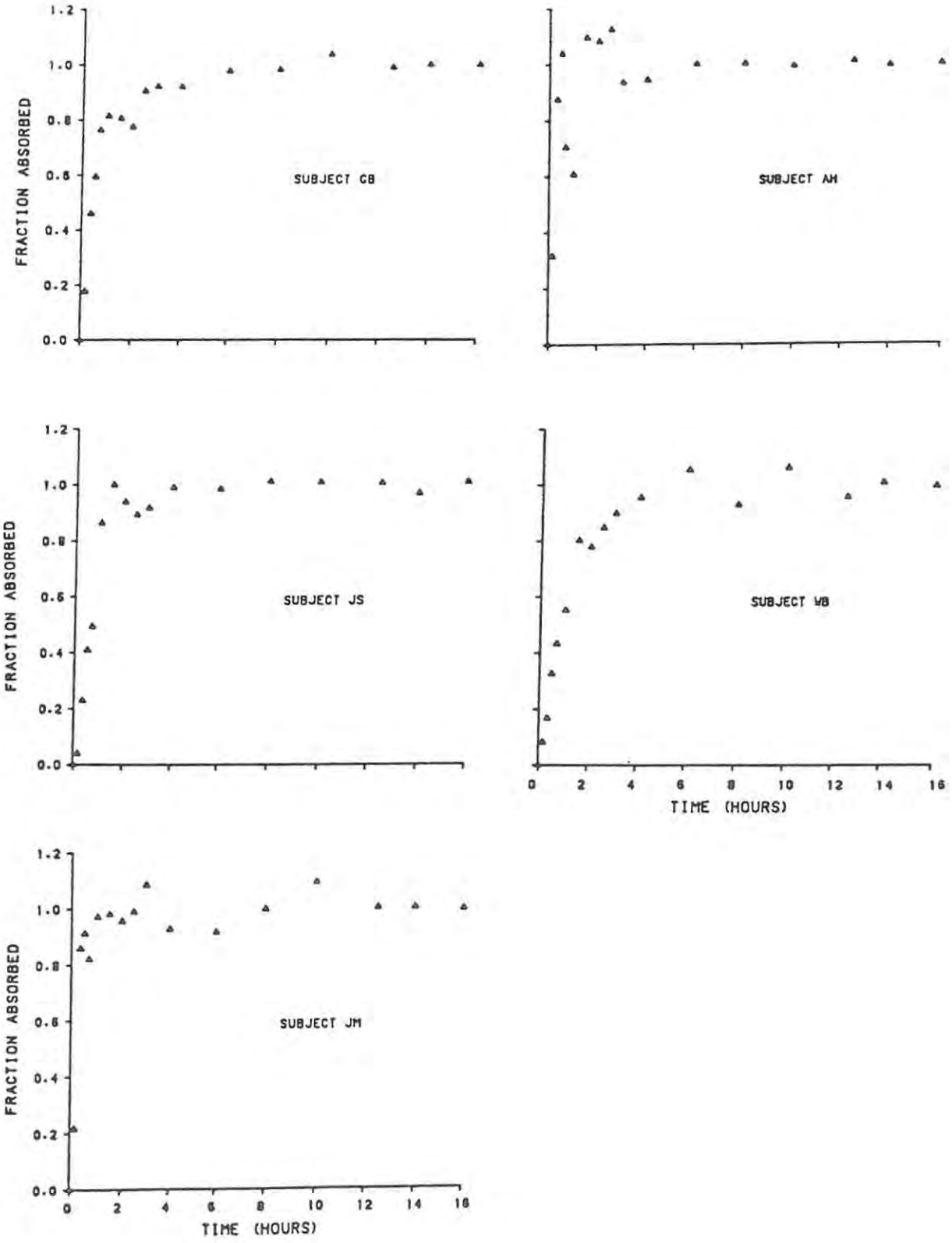
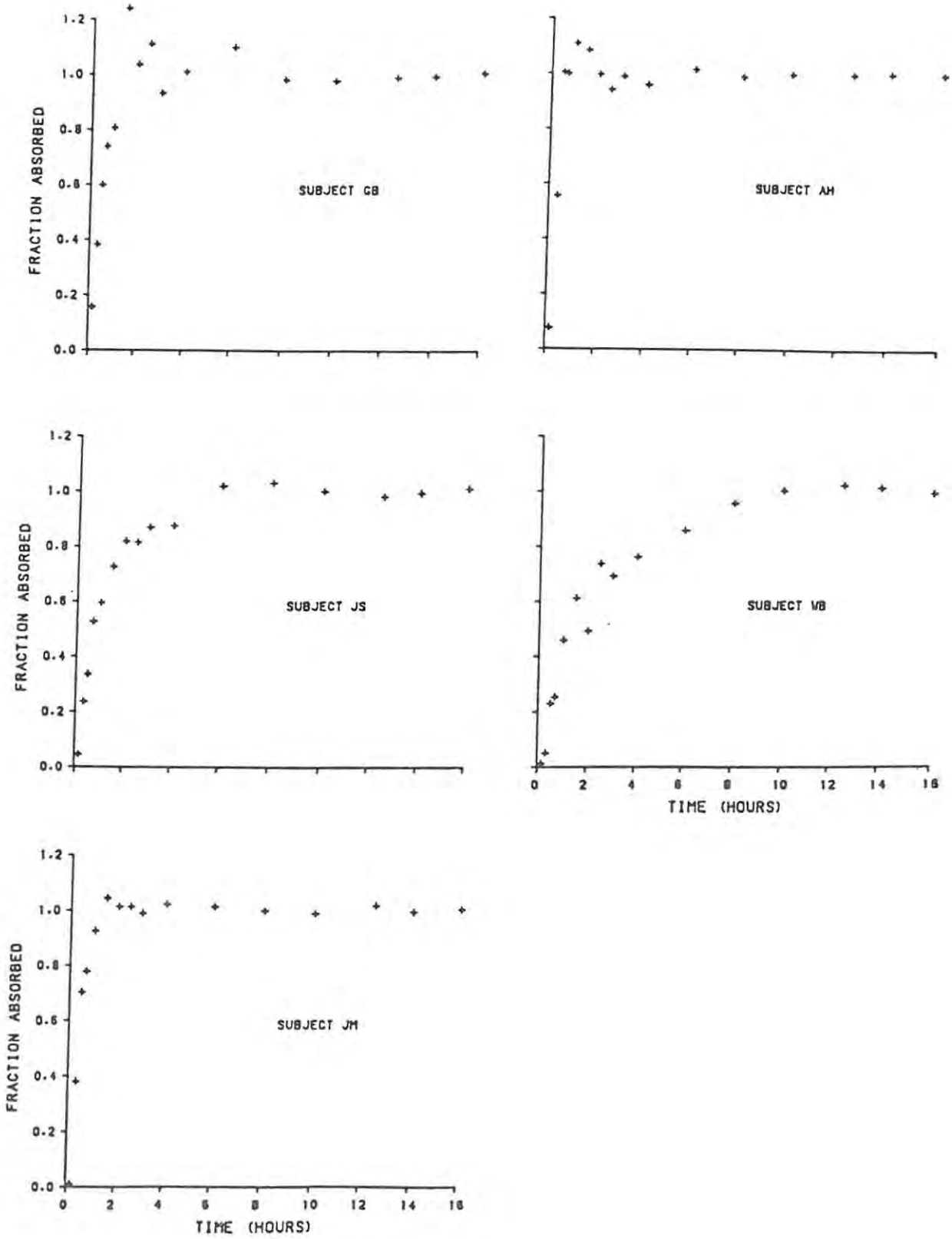


FIGURE 6.5 Wagner-Nelson absorption plots of serum concentration-time curves after administering the 100 mg PPA.HCl solution.



6.4.3 Renal Clearance

The renal clearance of a drug is that volume of blood that is cleared of the drug per unit time via the kidneys. Drugs may be eliminated from the systemic circulation by different pathways i.e., into the urine, bile, intestines, saliva, alveolar air, sweat and milk. The two major pathways of excretion are via the kidneys into the urine and via the liver into faeces. Drugs are usually filtered through the glomerulus in a non-selective process if they are in free, non-protein bound form. If a drug is not bound to plasma protein it is filtered in an amount equal to:

$$F = Cp.\ GFR\ (mg/min) \qquad (6\text{-}11)$$

where F = amount of drug filtered through the glomeruli (mg/min)
Cp = drug concentration in plasma (mg/mL)
GFR = glomerular filtration rate = renal clearance (mL/min)

The rate of filtration of many drugs can be retarded due to their binding to high molecular weight plasma proteins which are not filtered. The reabsorption of weakly acidic or basic drugs may be highly dependent on urine pH, since the relative amounts of the ionized and unionized form of a drug would vary with changes in urine pH. The urinary pH in man varies between pH 4.8 and pH 7.5 with an average of 5.8, and a significant change can be expected if a basic drug has a pKa between 7 to 11 i.e. renal clearance will increase in acidic urine, and conversely decrease in basic urine. Additional factors which may influence the extent of reabsorption of a drug from the distal tubules is urine flow rate and the functional integrity of the kidneys. Elimination of a drug may depend on hepatic and renal blood flow, hepatocellular enzyme activity and renal secretion (active).

The effect of altered binding on renal clearance of a drug depends on its extraction ratio. For a drug that is virtually all removed as it passes through the eliminating organ, the extraction ratio approaches 1 and clearance approaches its maximum value, organ blood flow. If the extraction ration is high, renal clearance depends upon blood flow

and not upon plasma binding. Conversely, if the extraction ratio is low, renal clearance is sensitive to plasma binding. Phenylpropanolamine has a high extraction ratio and is eliminated almost exclusively by the kidney. An increased hepatic and renal blood flow can thus be produced by increased cardiac output.

Interpretation of urine data with regard to levels of drug in the body is complicated for drugs whose renal clearance varies. The slight increase in renal clearance in the multiple dose study may be attributed to increased cardiac output by the sympathomimetic stimulatory effects of PPA. Sympathomimetic stimulation affects both the heart and the systemic circulation. It makes the heart a stronger pump, it increases the mean systemic pressure and increases the resistance to venous return. Approximately 25% of the cardiac output, 1.2-1.5 litres of blood per min., goes to the kidneys and renal blood flow is increased by increased cardiac output.

An interesting observation may be made with regard to renal clearance of weak bases. At low urine pH the renal clearance approaches the renal blood flow and such a clearance value usually suggests active secretion. Active secretion is inferred when the rate of excretion exceeds the rate of filtration of a drug, especially weak bases. Some reabsorption can occur but it is less than secretion. The secretory processes are located predominantly along the proximal tubule although these transport systems lack a high degree of specificity. Secretion can be so extensive that virtually all the drug in the blood is removed whether or not bound to plasma protein or located in blood cells.

When studying the pharmacokinetics of PPA, a weak base, it was found to be important to consider the pH of urine. Urine pH readings monitored throughout the trials (listed in Table A1) were found to fluctuate between 5.16 and 7.50. Phenylpropanolamine, being a weak base with a pKa of 9.4 would not undergo a significant change in tubular reabsorption at these values.

Renal clearance may be estimated in several ways

1. The average of the ratios of $\frac{d\ Ae/dt}{C}$ (6-12)

2. The slope of the plot of d Ae/dt versus C at mid-points of excretion intervals.

where d Ae/dt is the amount of drug (mg) eliminated per unit time (hr), CL is the renal clearance (L/hr) and C is the concentration of the drug in serum/plasma (ng/mL). Linear kinetics occurs only when clearance is constant, with the rate of elimination being directly proportional to concentration.

The semilog plots of serum concentration versus time for 25, 50 and 100 mg solution studies are shown in Fig. 6.6., and those for the multiple dose solution study are shown in Fig. 6.7. From these curves, it is difficult to extract any definite characteristics and no predictions concerning the presence of Michaelis-Menten kinetics could be made from these graphs.

FIGURE 6.6 Semilogarithmic plots of serum concentrations versus time after the ingestion of a 25 mg (Θ), 50 mg (△) and 100 mg (+) solution of PPA.HCl.

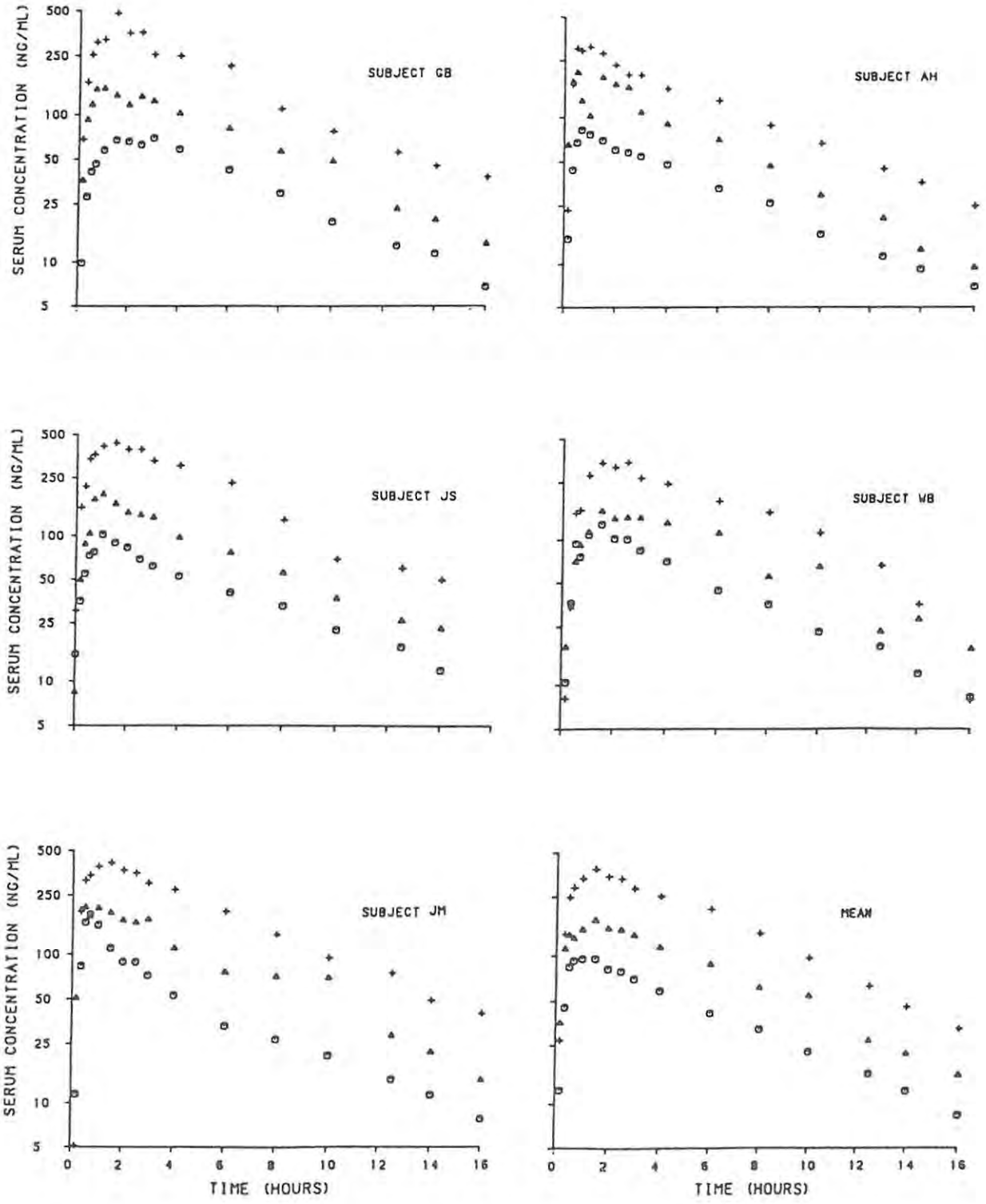
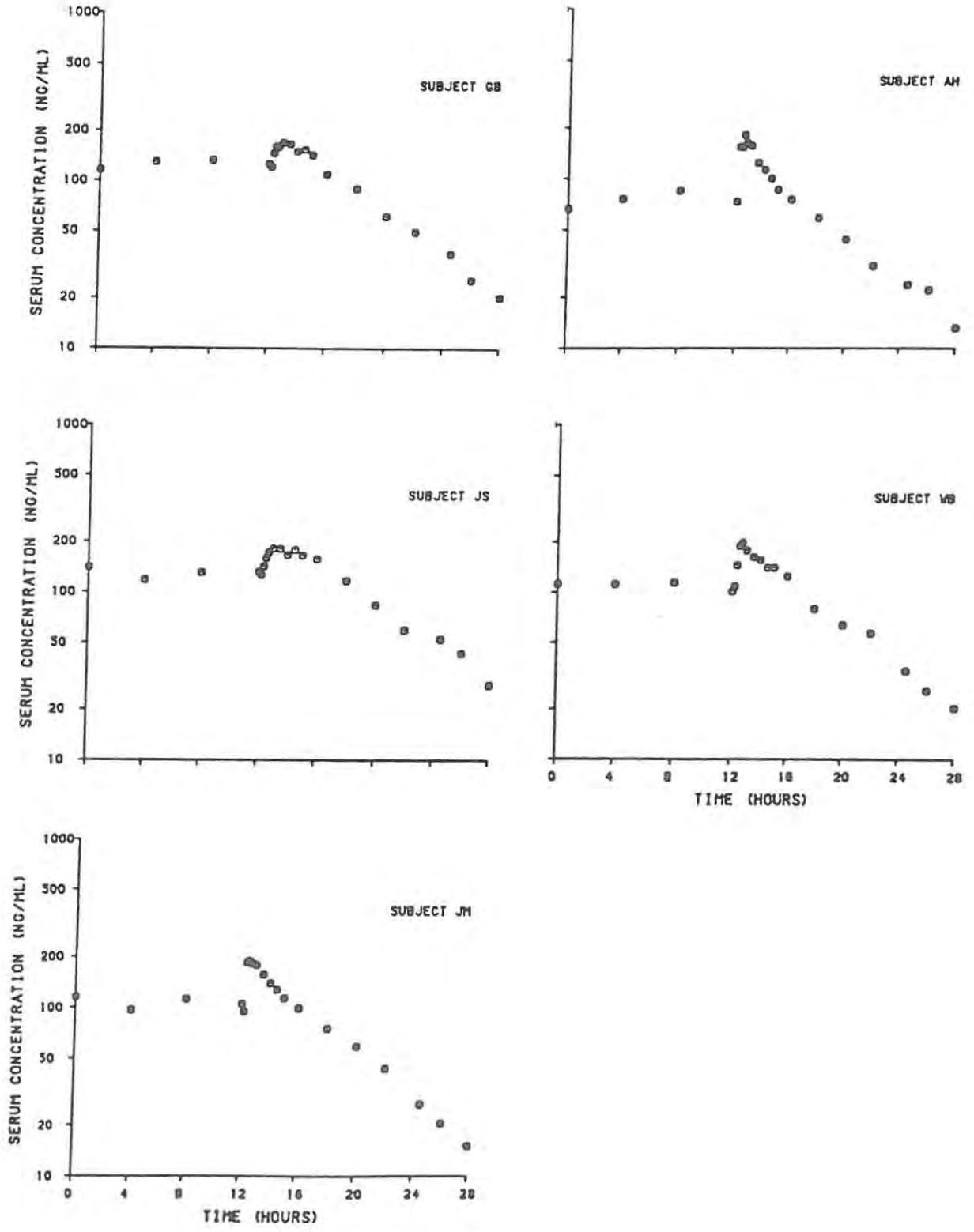


FIGURE 6.7 Semilogarithmic plots of serum concentrations versus time of the multiple dose study.



The relationship between renal clearance and serum concentrations in this study was determined under dynamic conditions, as serum concentrations of PPA were changing rapidly. Usually, relatively constant serum concentrations are maintained by continuous infusion of the drug. The point arises whether clearance is an artifact due to distribution effects. Unfortunately, volume of distribution could not be calculated due to lack of intravenous data. Distribution volumes and clearance, however, are usually separate and non-interacting pharmacokinetic elements.

Renal clearance was calculated for each time interval using Eq. 6-12, and the results can be seen in Table 6.6-6.9. The graph of urinary excretion rates versus the serum concentration at the mid-point of the urine collection interval was then plotted, the slope of which gave renal clearance values over the entire duration of the trial (see Fig. 6.8).

TABLE 6.6 Clearance values from the 25 mg solution study.

Time (hrs)	CL (L/hr)				
	GB	AH	JS	WB	JM
0.5	50.42	20.23	16.67	10.79	13.48
2	36.01	27.22	17.88	14.29	35.47
4	32.13	27.19	26.27	34.16	41.42
6	34.79	33.35	25.17	34.48	38.68
8	29.43	34.46	21.99	22.13	23.85
10	33.35	37.21	21.69	65.87	25.01
12.5	19.22	36.38	13.74	4.03	0.46
Mean	33.62	30.86	20.49	26.54	29.59
±SD	9.27	6.18	4.58	20.78	10.64
Graph*	37.09	32.50	21.80	20.07	22.61

TABLE 6.7 Clearance values from the 50 mg solution study.

Time (hrs)	CL (L/hr)				
	GB	AH	JS	WB	JM
0.5	17.31	7.05	22.59	35.63	14.11
2	21.36	21.10	16.33	25.75	27.35
4	46.75	17.51	31.35	22.08	36.96
6	26.63	41.74	18.36	47.25	31.86
8	28.49	46.25	32.02	29.14	30.15
10	33.64	23.05	25.05	12.85	37.93
12.5	26.63	61.12	31.36	56.85	31.77
Mean	28.69	31.11	25.29	32.79	30.01
±SD	9.51	19.02	6.51	15.11	7.93
Graph*	21.80	28.20	20.01	25.01	29.63

TABLE 6.8 Clearance values from the 100 mg solution study.

Time (hrs)	CL (L/hr)				
	GB	AH	JS	WB	JM
0.5	-	31.03	17.32	17.80	13.20
2	26.29	47.30	25.11	19.45	20.27
4	73.30	43.25	20.22	26.28	26.55
6	16.17	40.34	32.91	21.93	39.32
8	17.28	68.18	22.78	27.85	25.59
10	26.59	85.96	29.44	34.95	32.82
12.5	22.96	41.23	25.91	33.42	24.39
Mean	30.43	51.04	24.81	25.95	26.02
±SD	21.50	19.14	5.32	6.65	8.40
Graph*	26.5	41.8	24.5	22.6	21.9

FIGURE 6.8 Plots of urinary excretion rate versus serum concentration at the mid-point of the urine collection interval after the ingestion of a 25 mg (o), 50 mg (Δ) and 100 mg (+) solution of PPA.HCl.

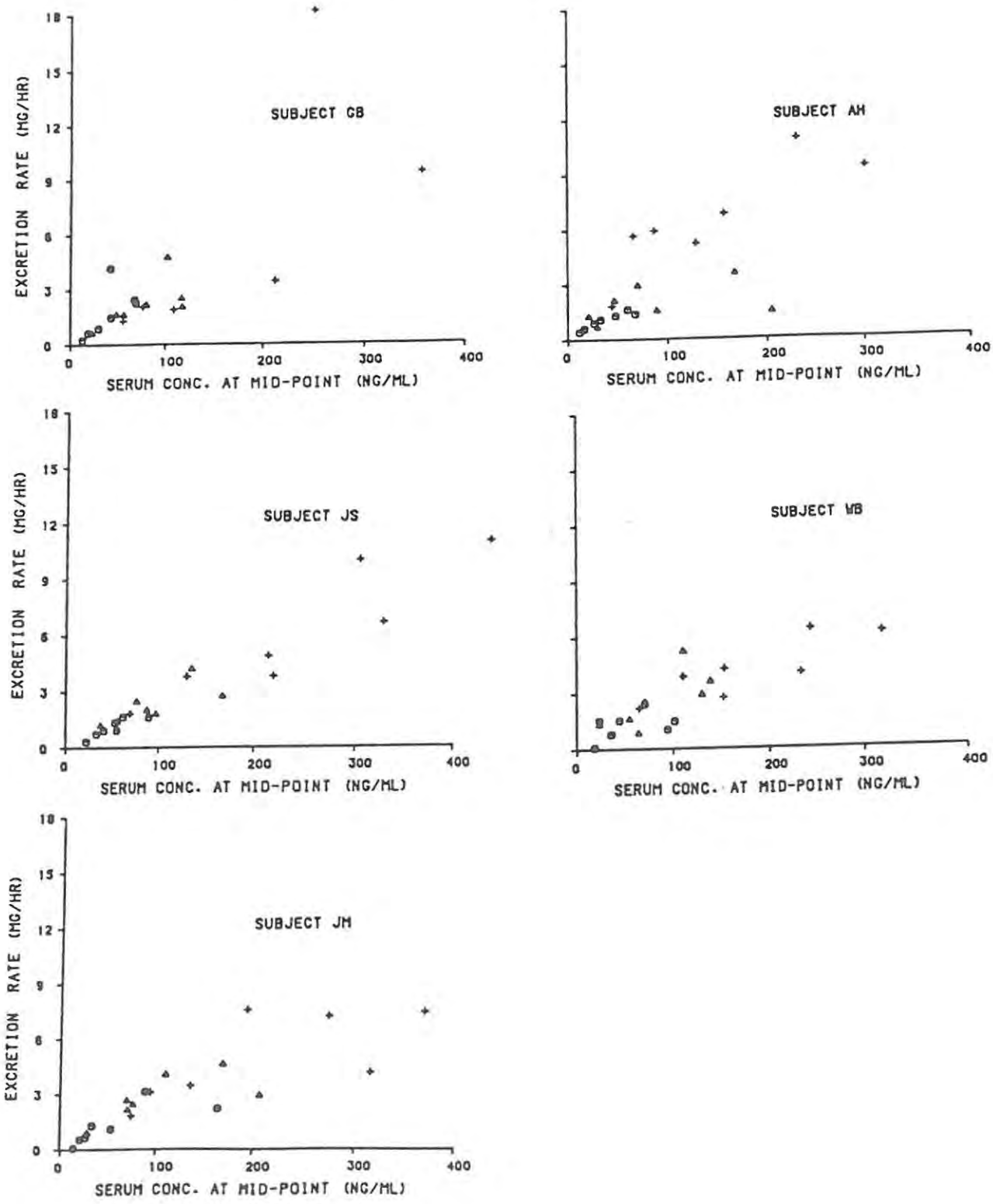


TABLE 6.9 Mean clearance values from the 25, 50 and 100 mg solution studies.

	CL (L/kg/hr) Mean Data		
	100mg	50mg	25mg
GB	0.52	0.48	0.57
AH	0.60	0.38	0.38
JS	0.41	0.42	0.34
WB	0.32	0.42	0.34
JM	0.35	0.40	0.40
Mean	0.44	0.42	0.41
±SD	0.11	0.03	0.09

* The excretion rates were plotted against the serum concentrations at the mid-points of the excretion intervals and renal clearance was obtained from the slope of this line.

From the multiple dose study, the apparent renal clearance of PPA was calculated from the 4 hour dose interval after the last dose using the linear trapezoidal rule:

$$CL = \frac{\text{Dose (p.o.)}}{AUC_{0-4(p.o.)}} \qquad (6\text{-}13)$$

where the Dose is in µg. The results can be seen in Table 6.9. The seemingly critical assumption in the approach outlined above (aside from the assumption of linearity) is that clearance remains the same between treatments in the same individual. This assumption can only be an approximation, as intra-subject variability in drug disposition is well documented. It follows that there is interest in reducing the effect of intra-subject variability in bioavailability studies which could be accomplished if differences in systemic clearance (CL) in the

same individual from one treatment to another is accounted for. Unfortunately, this is usually not possible because one cannot determine CL after oral administration without making some assumption concerning bioavailability. An alternative approach is based on differences in drug half-lives (291).

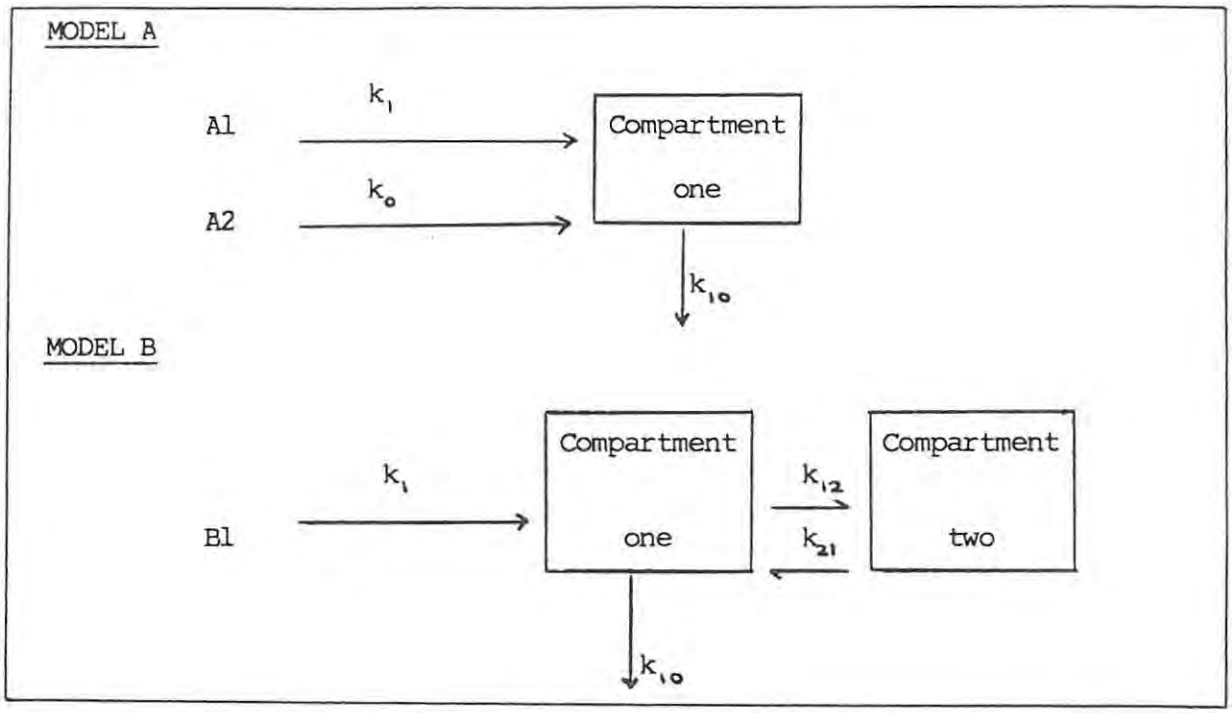
TABLE 6.10 Clearance values from the multiple dose solution study

Subject	Clearance(L/hr)	Clearance(L/kg/hr)
GB	42.53	0.72
AH	54.22	0.64
JS	37.86	0.62
WB	41.32	0.52
JM	45.42	0.61
Mean	44.27	0.62
±SD	6.18	0.07

6.5 MODELLING OF DATA

The serum concentration-time data obtained following single dose oral administration of the solutions were fitted to appropriate linear pharmacokinetic models (see Fig. 6.9). The digital computer, nonlinear regression analysis programme (NONLIN) of Metzler (292) was used to attempt the various fits, with the DFUNC subroutine being modified for each function, and equal weighting of the data points. The three models used in this study include a conventional one-compartment open model with either apparent first-order absorption and linear elimination (Model A1), or apparent zero-order absorption and linear elimination (Model A2), and a two-compartment model with continuous first-order absorption and linear elimination (Model B1). Each set of data were fitted individually.

FIGURE 6.9 Pharmacokinetic models for phenylpropanolamine.



In all the following equations describing rate of change in drug concentration in the body, the bioavailability term, F, was assumed to equal 1.0 (See Section 6.4.1).

Symbols are defined as follows:

dC/dt	=	rate of change of drug concentration in the central compartment
k_a	=	first order absorption rate constant
D	=	dose of drug
V	=	volume of central compartment
A_2	=	amount of drug in peripheral compartment
C_1	=	concentration in the central compartment
K_{10}	=	elimination rate constant

K_{12} = first order rate constant for transfer of drug from the central to the peripheral compartment

K_{21} = first order rate constant for transfer of drug from the peripheral to the central compartment

t = time after drug administration

t_0 = lag time between drug administration and appearance of drug in the serum

k_0 = apparent zero absorption rate constant

T = a constant corresponding to the absorption time, after absorption apparently ceases. (During absorption, T is a variable and equal to t)

The first model incorporated a one body compartment model (1BCM), a first order absorption rate constant and linear elimination. The differential equation describing the model is shown below:

$$\frac{dC}{dt} = k_a \frac{D}{V} e^{-k_a(t-t_o)} - K_{10}C_1 \qquad (6\text{-}14)$$

The lag time (t_{lag}) was introduced to accommadate the delay in onset of PPA absorption.

The data were then fitted to a 2BCM with continuous absorption and linear elimination. The 2BCM was included in an attempt to account for the observed rapid decrease in concentration which occurred just after the peak. The differential equation describing the rate of change of drug in the peripheral and central compartments are shown in Eq. 6.15.

$$\frac{dC}{dt} = k_a \frac{D}{V} e^{-k_a(t-t_o)} + K_{21}\frac{A_2}{V} - K_{12}C_1 - K_{10}C_1 \qquad (6\text{-}15)$$

The interpretation of PPA absorption as zero order input was assessed by fitting the data to the following equation:

$$C = \frac{k_o(e^{k_{10}T} - 1)e^{-k_{10}t}}{Vk_{10}} \qquad (6\text{-}16)$$

Goodness of fit of data to Models A1, A2 and B1 was assessed by calculating the correlation coefficient for each data set of observed and predicted data, and by visual observation of the curves. In pharmacokinetic analysis of the time course of plasma concentration of a drug, it is important to determine the number of parameters in a pharmacokinetic model. Akaike's Information Criterion (AIC) was thus used to select an appropriate model (293) with the equation having the minimum AIC regarded as the best characterizing the data set.

$$AIC_j = n\ln RE_j + 2p \qquad (6\text{-}17)$$

where n is the number of observations, p, the number of parameters, Re, the weighted sum of squared deviations and j represents the jth data set. It should be remembered however, that the minimum AIC estimation does not select the "correct" model or equation but rather the simplest representation of a particular set of pharmacokinetic data.

6.6 RESULTS

The observed values and the resultant fits to models A1 and A2 can be seen in Figs. 6.10-6.12. In Appendix 2, the observed and predicted values for serum data from the 25, 50 and 100 mg solution studies resulting from the fits to models A2 and B1 are shown in Tables A2.1 and A2.2. The parameter values for all three models are depicted in Table A3.1. Observed and resultant fits to model B1 can be seen in Figs. 6.13-6.15. The results of AIC values for individual subjects are shown in Table 6.11.

FIGURE 6.10 Observed data and predicted fits to the 25 mg serum data using models A1 and A2.

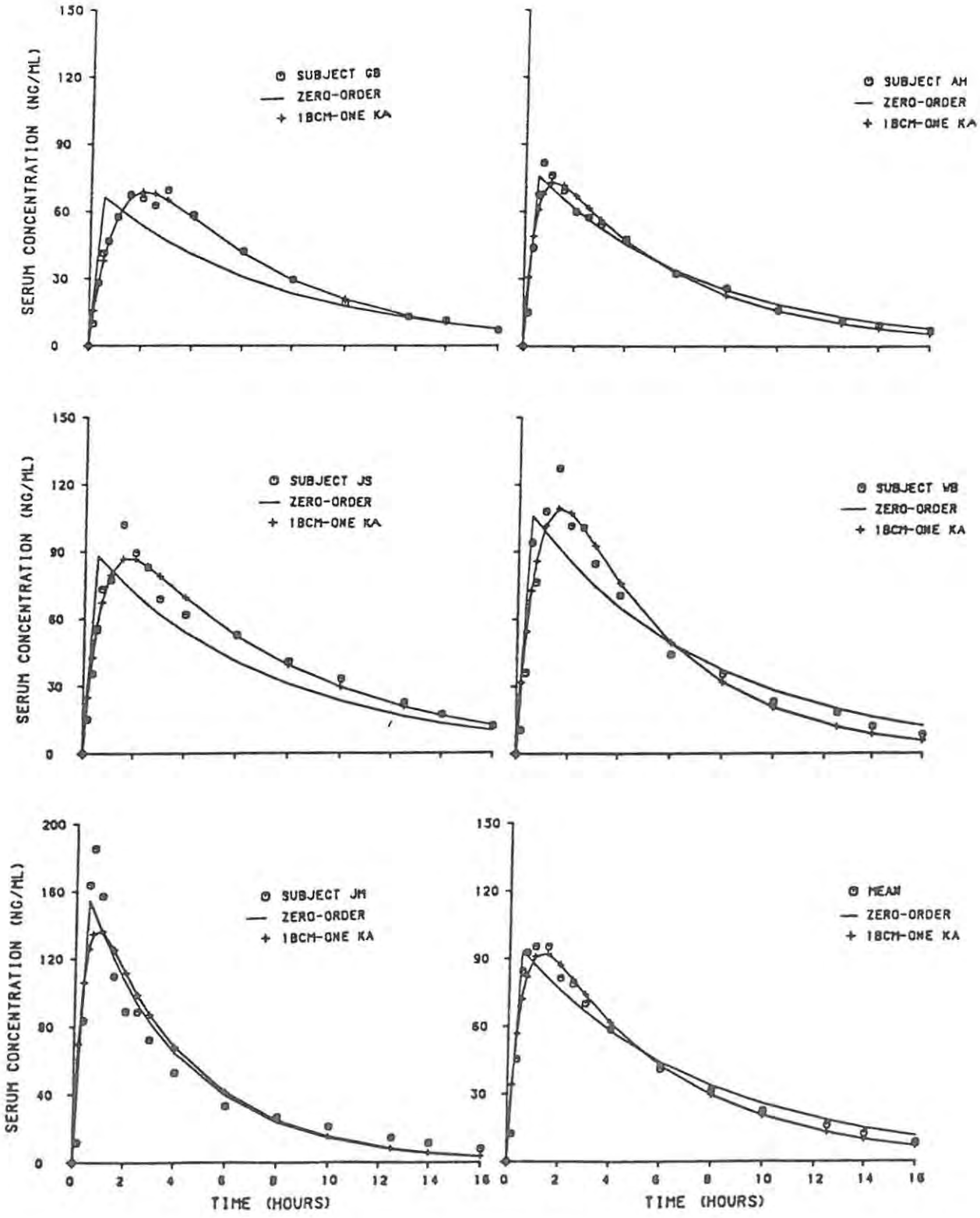


FIGURE 6.11 Observed data and predicted fits to the 50 mg serum data using models A1 and A2.

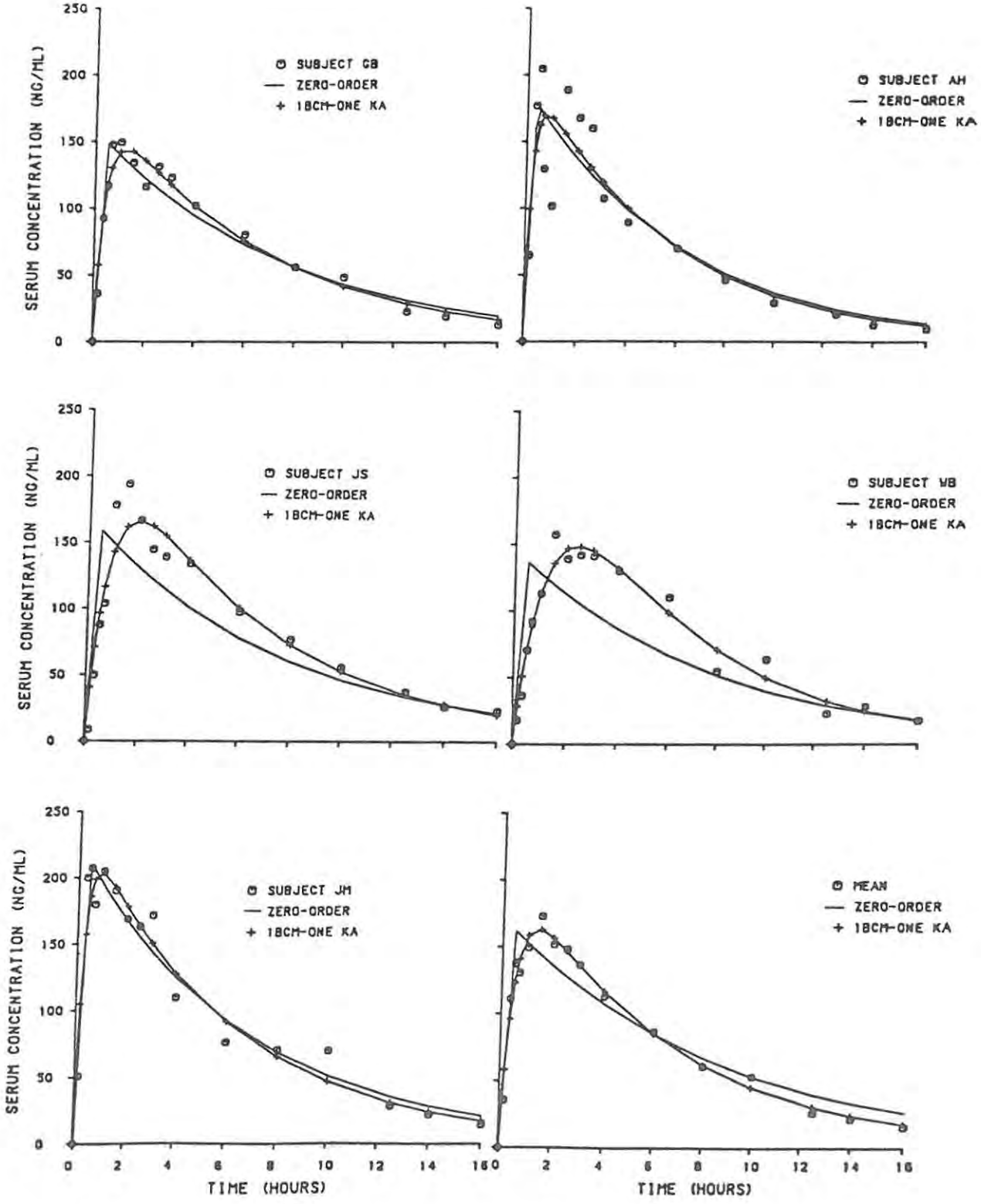


FIGURE 6.12 Observed data and predicted fits to the 100 mg serum data using models A1 and A2.

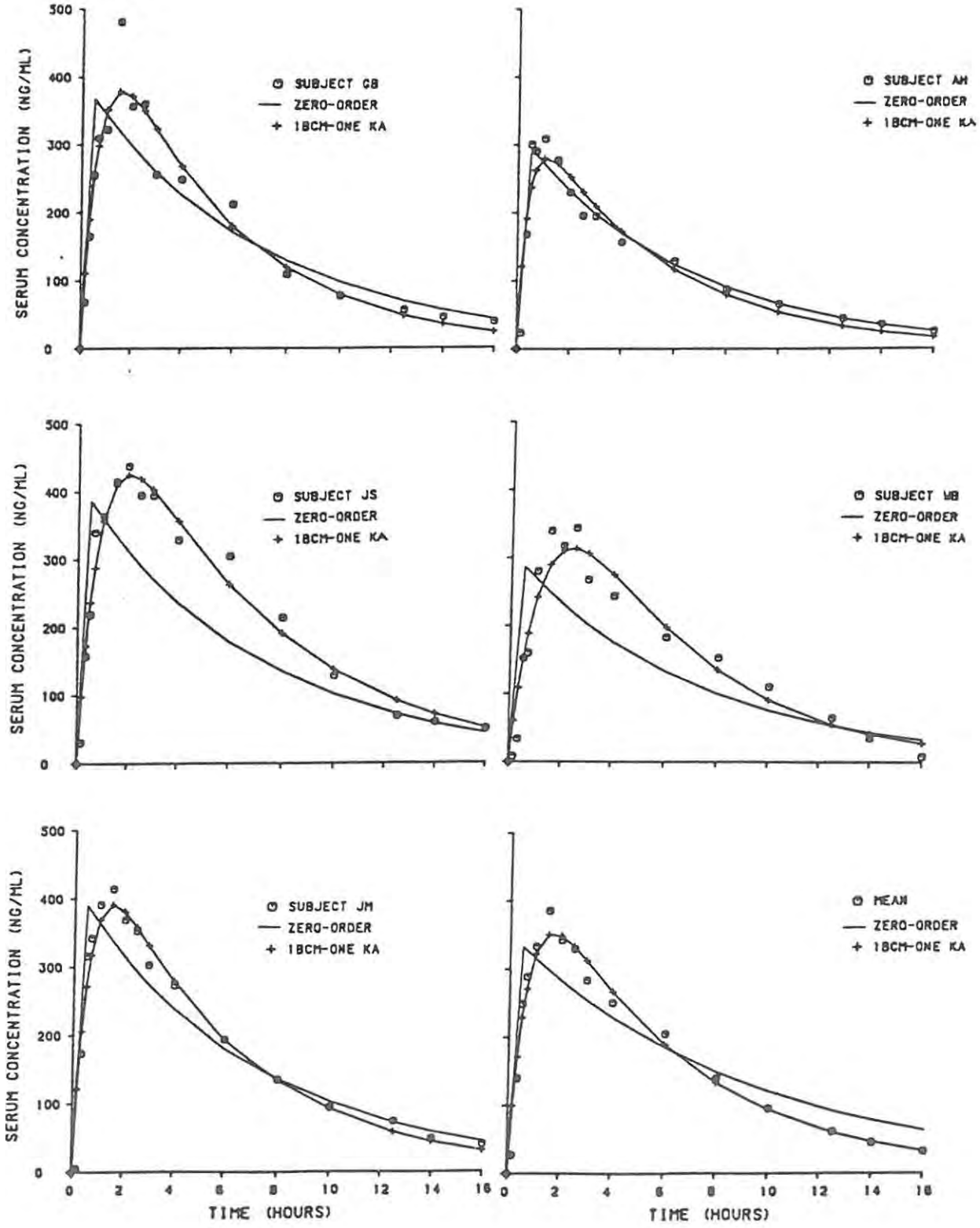


FIGURE 6.13 Observed data and predicted fits to the 25 mg serum data using model B1.

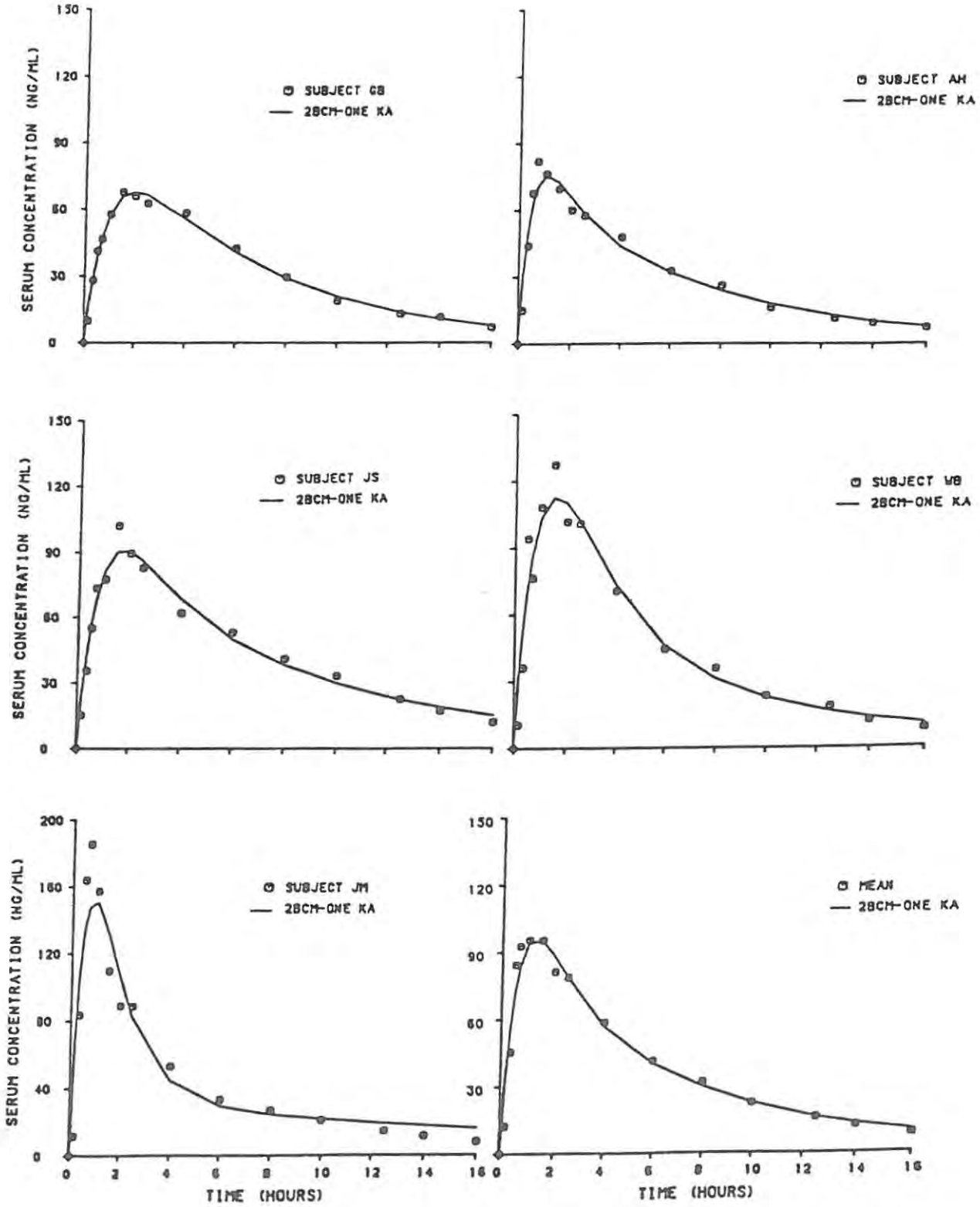


FIGURE 6.14 Observed data and predicted fits to the 50 mg serum data using model B1.

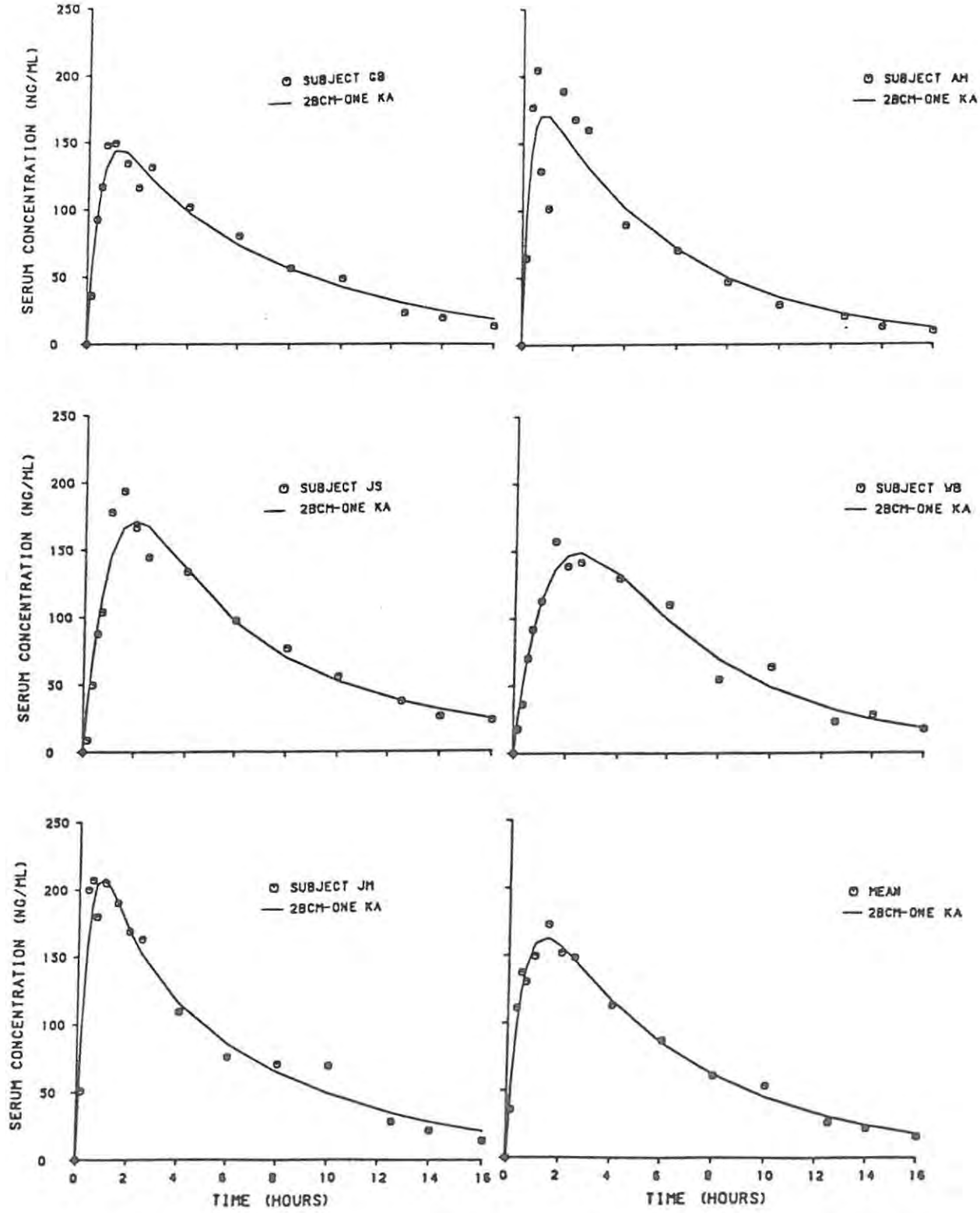
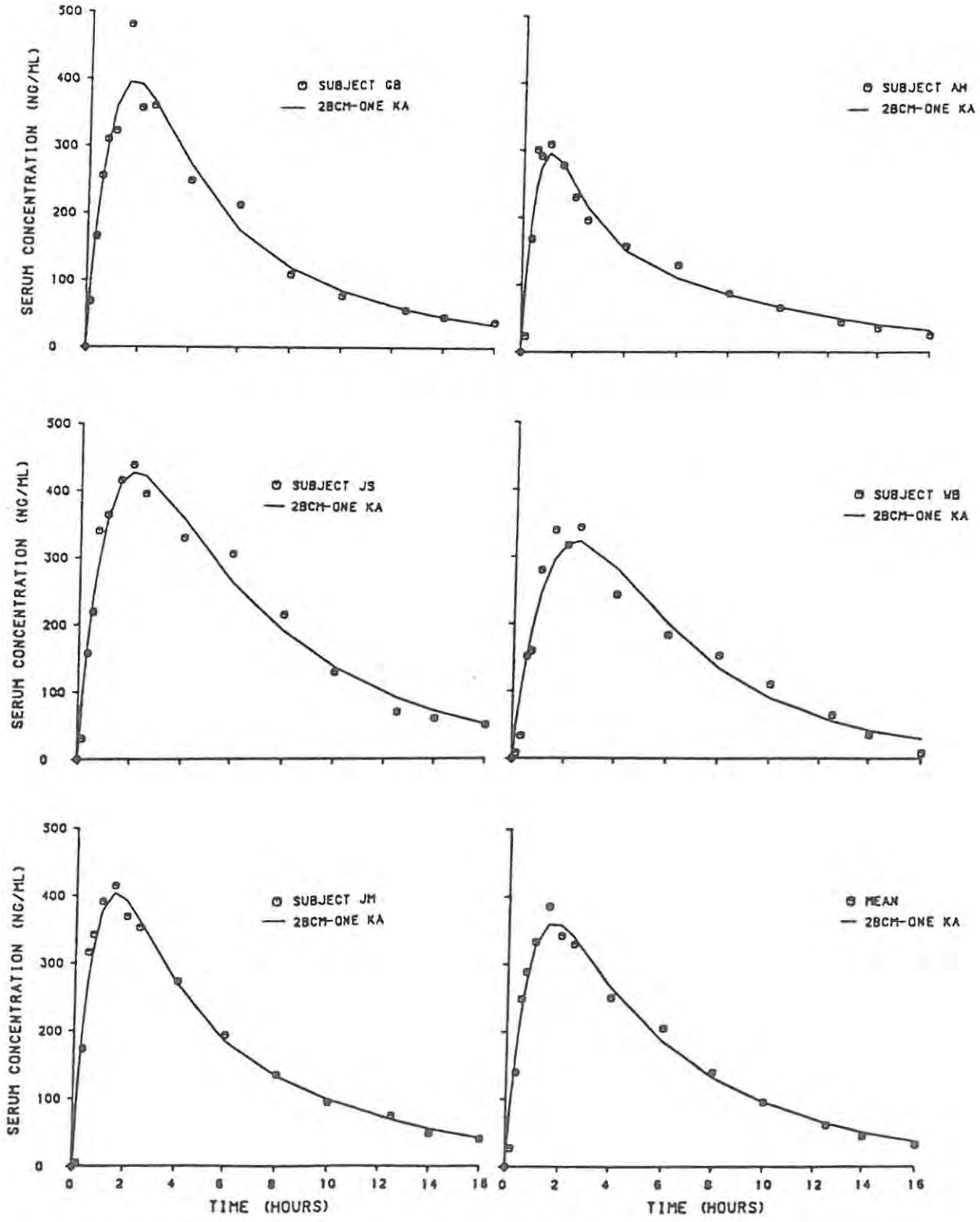


FIGURE 6.15 Observed data and predicted fits to the 100 mg serum data using model B1.



The 1BCM with first order absorption (Model A1) was unable to account for the rapid absorption phase and resulted in a large over-estimation of initial concentrations followed by an inability to attain the high peak concentrations. The predicted fit to the entire curve was poor and exhibited a more rounded peak which was unable to account for the rapidly decreasing values tailing off to a gentle slope in the elimination phase. The value of r for the mean serum fits were 0.974 (25 mg study), 0.987 (50 mg study) and 0.983 (100 mg study).

A rapid increase in drug concentration after oral administration followed by a fairly rapid decline is reported to be characteristic of zero order absorption (294). Therefore, a model incorporating zero order absorption was used in an attempt to improve characterization of the absorption and immediate post-absorption phases. This however, did not result in an improvement of fit as indicated by the correlation coefficients (which decreased from a mean of 0.981 to 0.952), AIC values and visual observation.

The fit for the 2BCM with first order absorption (Model B1), as indicated by the values for r, (0.98, 0.99, 0.99) was better than the fit for the 1BCM (Model A1), with an improved fit of the latter part of the curve. In general, however, the absorption phase was still poorly characterized by an inability to attain the high peak concentrations. A slight improvement of fit, as indicated by the correlation coefficients, AIC values and visual observation resulted from Model B1.

The pharmacokinetics of PPA after oral administration has previously been described by a two compartment model with discontinuous absorption (290). Our studies indicate that the two compartment model (Model B1) adequately described the serum data of PPA after oral administration. The 1BCM also described PPA plasma data well but fitted poorly in some subjects (JM, WB and JS) as evidenced by over-estimation during earlier doses. Simple two compartment distribution with linear formation and elimination seems to adequately describe the disposition of PPA in these subjects.

TABLE 6.11 AIC values for individual subjects

DOSE (mg)	MODEL	SUBJECTS					
		GB	AM	JS	WB	JM	Mean data
25		87.67	117.06	117.14	136.60	164.34	125.36
50	A1	131.51	169.05	151.40	133.18	158.96	130.78
100		176.97	174.92	168.27	174.59	176.07	164.05
	Mean	132.05	153.67	145.60	148.12	166.15	140.06
25		142.98	109.02	148.18	152.72	152.99	127.99
50	A2	139.45	165.80	177.24	176.21	153.40	149.31
100		196.36	164.08	207.94	205.88	190.69	189.48
	Mean	159.59	146.30	177.78	178.27	165.65	155.0
25		82.64	111.71	107.76	137.99	162.91	119.48
50	B1	127.39	172.80	153.16	129.73	151.50	134.49
100		164.67	165.12	162.94	166.88	168.06	156.32
	Mean	124.90	149.87	141.28	144.86	160.82	136.76

After intake of PPA solution, a double peak phenomenon was observed. The irregular profile or hump is a complicating factor in the interpretation of the data. However, during the conduct of the study a publication appeared (300), which suggested a more flexible way to correct for this phenomenon according to a double-site model for drug absorption as a determinant of kinetic parameters. The use of multi-fraction absorption models where drugs in the gastrointestinal tract are assumed to be divided into several fractions, each with its respective lag time and absorption rate is described by Murata *et al.* (295). The choice of a suitable kinetic model requires decisions with respect to both the compartmental and absorption characteristics of

the drug. Discontinuous absorption characteristics may be inferred from a graphical representation of experimental data or from poor computer fits obtained using continuous absorption models.

6.7 DISCUSSION

A number of digital computer programs are routinely employed in pharmacokinetics for the nonlinear least squares estimation of pharmacokinetic parameters and for the simulation of the time course of the drugs in pharmacokinetic systems. Several programs, e.g., NLIN (296), SAAM (297), and NONLIN (292), are available for the nonlinear least squares regression analysis of pharmacokinetic systems. Of all three programs, NONLIN appears the most versatile in that either intergrated or differentiated equations can be employed, more than one set of data can be fitted simultaneously and it can be employed for simulating data. In addition, zero order processes and nonlinear pharmacokinetic models can be fitted employing this program.

A frequent problem in nonlinear least squares fitting of observed data to a mathematical model is that the so-called "least squares parameter estimates" are dependent upon the initial estimates of the parameters, which are part of the input data provided to the digital computer. The lack of convergence to the same set of least squares estimates of the parameters may be caused by the lack of consistency of the parameters from one treatment to the next as is assumed in simultaneous fitting. Simultaneous fitting, however, has the distinct advantage of reducing standard deviations of the estimated parameters. Lack of convergence to the same set of least squares parameter estimates when the initial parameter estimates are changed is also partly caused by 1) error in data 2) the model not being appropriate for the data, and 3) convergence to a local minimum on the least squares surface rather than the true minimum.

Drug absorption from the gastrointestinal tract is generally considered to occur by passive diffusion through the gastrointestinal membrane. Some exceptions have been described where active processes are involved (298), and first order absorption is unable to depict correctly the appearance of the drug in the blood stream. The same occurs for drugs that exhibit enterohepatic recycling for which particular pharmacokinetic models have been developed (299). For such drugs, reabsorption leads to non-monotonicity in the shape of plasma concentration curves with the appearance of successive peaks. Other physiological phenomena may explain such curves. Non-homogenous absorption of drugs through the gastrointestinal membrane could lead to a similar multiple peak phenomenon. This phenomenon has been observed and interpreted accordingly to a double-site model for drug absorption (300) where the concept of an "absorption window" by Kubler (301) was developed.

In addition, discontinuous absorption models (302) have been proposed for the analysis of plasma drug concentration data with irregular absorption, in which the absorption rate constant is variable through the process of absorption. It has been shown that Metzler's NONLIN program can be used for fitting these discontinuous absorption profiles. The absorption phase of PPA from the use of a biphasic, discontinuous absorption model has been well-characterized by previous workers (290) using serum data after the administration of a solution of PPA.

A previous report (303) described a digital computer method which utilized all serum concentration data collected during repetetive dosing studies to estimate pharmacokinetic model constants and to fit the entire time course of drug concentration in serum/blood. This method can be used for detecting changes in pharmacokinetic model constants and for characterizing Michaelis-Menten kinetics during multiple dosing and accomodates changes in dose or dosing interval during a multiple dose regimen. Several investigations found that pharmacokinetic parameters of certain drugs change in a systematic way during repetetive drug administration (41-43). Self-induction, self-

inhibition and product inhibition of metabolism are possible causes of variation in elimination rate constants. Under certain conditions, a drug subject to capacity-limited metabolism will demonstrate pharmacokinetic characteristics similar to that of a drug subject to self-inhibition or product-inhibition i.e., the half-life will appear to increase during multiple dosing. Variability in absorption rate constants and/or the amount absorbed as well as changes in the apparent volume of distribution from dose to dose may also occur during a repetitive dose regimen.

CHAPTER 7

CONCLUSIONS AND DISCUSSION

Although extensively used, there is little pharmacokinetic information on PPA disposition in the literature, possibly due to the difficulties inherent in the determination of PPA especially at low concentrations encountered in the blood after the administration of oral dosage forms. The HPLC method described using UV detection at 210 nm has been shown to be precise, specific and sufficiently sensitive so that reliable measurements of PPA concentrations may be obtained for serum and urine for up to 16 hours following a 25 mg oral dose, and demonstrates that the method is adequate for use in pharmacokinetic and bioavailability studies. Previous HPLC methods used to determine PPA in biological fluids are relatively time consuming with respect to sample preparation or relatively insensitive to detection of low concentrations in serum, and the method described here is rapid and relatively simple. The general approach presented produced very clean chromatograms and thus should be considered for other primary amines.

The potential for an adverse hypertensive effect following the use of PPA has, over the years, been the topic of sporadic case reports and studies. Some of these reports are anecdotal while others refer to situations where frank overdoses and/or multiple drugs, including PPA, were used (44, 82, 84, 85, 244). Our findings, however, were consistent with other reports and controlled clinical studies in the literature (80, 245) that found no significant changes in the blood pressure and pulse rate in individuals with normal blood pressure receiving PPA. It was observed that PPA increased blood pressure in a dose-related fashion with significant inter-subject variability. Some volunteers reported side-effects although these were considered by the investigator to be minor.

Phenylpropanolamine is marketed as a racemic mixture of two enantiomers, and in this study, the pharmacokinetic properties of the racemic mixture were examined. To pursue the possibility of stereoselective differences in the binding in plasma and in the disposition of PPA, further studies could be carried out to examine the pharmacokinetic properties of d- and ℓ- norephedrine.

Bioavailability parameters were calculated from the *in vivo* solution data by use of noncompartmental methods, which do not require the assumption of a particular compartment model. Results obtained from noncompartmental methods are more physiologically significant than those obtained from methods using empirically derived models. Subjects AH and WB consistently displayed rapid absorption rates. Both subjects were of average weight and age for this study and discrepancies can only be attributed to individual variation. The serum AUC values calculated for AH were, in all trials, relatively low. In contrast, the serum AUC values calculated for subject JS were relatively high. Subject AH was large in stature and had the highest body weight, and subject JS was small in stature, thereby possibly accounting for this phenomenon. Interestingly, subject JS displayed the highest changes in blood pressure and experienced more side-effects than the other subjects. Serum AUC values appeared to increase in proportion to the increasing dose administered for all 3 trials. Deviation from linearity, however, was noted in the C_{max} values. Renal clearance of the drug after administration of the solutions was also investigated. Renal clearance was found to be relatively constant throughout the trial, except in subject AH, where the renal clearance fluctuated, with higher values occurring at higher serum concentrations of the drug. The pharmacokinetic parameters estimated are in good agreement with previously reported parameters. The high clearance values as well as volume of distribution values, were similar to those of Dowse *et al.* (290).

No reliable protein binding data appear in the literature for PPA, protein binding of drug being a phenomenon which could account for higher clearance values at increased concentrations. Plasma protein

binding of PPA was investigated, and was found to be only slightly dependent on the concentration in blood: 4.2 ±1.1% at 95 ng/mL and 17.8 ±2.9% at 280 ng/mL. This small change in binding was regarded as irrelevant for the interpretation of pharmacokinetic data of PPA. As the renal clearance exceeds the glomerular filtration rate, active tubular secretion is a possible mechanism by which additional PPA may be excreted.

The solution data were fitted to compartmental models which were characterized by a series of differential equations. The inability of the 1BCM with apparent zero order absorption to describe the kinetics of PPA in the body was immediately apparent. The use of the 1BCM with apparent first order kinetics slightly improved characterization of the curve, but the best fit was obtained using a two compartment model which improved characterization in the distribution phase immediately after maximum concentration. In the absorption phase, however, the predicted peak for most subjects was slightly lower than the experimentally observed value. The elimination via linear kinetics was studied, and the 25, 50 and 100 mg solution data were well described by this process. Upon oral absorption, PPA was rapidly, but irregularly absorbed. Some subjects displayed irregular profiles of drug concentration serum profiles which were difficult to describe. However, this phenomenon could be interpreted according to a double-site model for drug absorption (300).

There have been no published reports in which intravenous data were used to estimate oral PPA bioavailability in humans. Bioavailability has been estimated from urinary PPA recovery since PPA is largely excreted unchanged in the urine. This method, however, could overestimate bioavailability in cases in which the drug is metabolized and the analytical method is not specific. The availability of intravenous data would enable the calculation of the absolute bioavailability of the drug, the volume of distribution, and the total clearance from the body. In computer modelling, known values such as volume of distribution obtained from an intravenous study could be incorporated as a constant, decreasing the number of parameters to be optimized.

APPENDIX 1

TABLE A1.1

Phenylpropanolamine Serum concentrations after the administration of 25 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	CONCENTRATION OF PPA IN SERUM (ng/mL)					MEAN
	GB	AH	JS	WB	JM	
0	0	0	0	0	0	0
0.17	9.86	14.96	15.24	10.39	11.48	12.39
0.33	28.07	43.89	35.45	36.07	83.49	45.39
0.50	41.28	67.48	54.90	93.99	163.81	84.29
0.67	46.65	81.81	73.19	76.33	185.25	92.65
1.00	57.62	76.10	77.36	107.99	157.18	95.25
1.50	67.47	69.44	102.01	127.16	109.72	95.16
2.00	65.76	59.92	89.42	101.52	88.96	81.13
2.50	62.64	57.46	82.97	100.68	88.70	78.49
3.00	69.39	54.13	68.78	84.62	72.28	69.84
4.00	58.33	47.73	61.78	70.41	53.00	58.25
6.00	42.25	32.50	52.76	44.23	33.09	40.97
8.00	29.39	25.91	40.79	35.47	26.83	31.68
10.00	18.74	15.96	33.10	23.00	20.87	22.33
12.50	12.84	11.27	22.56	18.20	14.39	15.85
14.00	11.31	9.21	17.20	11.90	11.29	12.18
16.00	6.74	6.95	11.90	8.20	7.78	8.31

TABLE A1.2

The cumulative urinary excretion (Ae) of PPA after the administration of 25 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	Ae (mg)				
	GB	AH	JS	WB	JM
0	0	0	0	0	0
0-1	4.16	1.37	1.00	1.02	2.21
1-3	9.01	4.63	4.11	3.91	8.44
3-5	13.47	7.22	7.36	8.72	10.64
5-7	16.41	9.39	10.02	11.77	13.20
7-9	18.14	11.18	11.81	13.34	14.47
9-11	19.39	12.36	13.25	16.37	15.52
11-14	20.13	13.59	14.17	16.59	15.54

TABLE A1.3

Phenylpropanolamine Serum concentrations after the administration of 50 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	CONCENTRATION OF PPA IN SERUM (ng/mL)					
	GB	AH	JS	WB	JM	MEAN
0	0	0	0	0	0	0
0.17	35.99	64.58	8.45	18.06	50.73	35.56
0.33	92.47	176.79	49.63	36.42	199.86	111.03
0.50	116.80	204.44	87.59	70.43	207.06	137.26
0.67	147.65	129.36	103.60	92.00	179.73	130.47
1.00	149.09	101.40	177.89	112.95	204.83	149.23
1.50	134.21	188.51	193.60	157.21	190.11	172.73
2.00	116.12	167.55	166.42	138.98	168.91	151.60
2.50	131.36	159.81	144.40	141.88	163.14	148.12
3.00	123.12	107.16	138.81	141.15	171.66	136.38
4.00	102.03	89.07	133.64	130.00	109.84	112.92
6.00	80.19	69.64	97.12	110.26	76.03	86.65
8.00	56.15	46.27	76.54	54.57	70.64	60.83
10.00	48.44	29.11	55.76	63.79	69.60	53.34
12.50	22.90	20.45	37.28	23.04	28.33	26.40
14.00	19.28	12.58	26.02	28.12	21.90	21.58
16.00	13.18	9.48	22.98	17.54	14.22	15.48

TABLE A1.4

The cumulative urinary excretion (Ae) of PPA after the administration of 50 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	Ae (mg)				
	GB	AH	JS	WB	JM
0	0	0	0	0	0
0-1	2.02	1.44	1.98	2.51	2.92
1-3	6.98	8.51	7.42	9.66	12.16
3-5	16.51	11.63	15.76	15.39	20.28
5-7	20.78	17.45	19.33	25.81	25.11
7-9	23.97	21.68	24.23	28.98	29.36
9-11	27.73	23.02	27.02	30.61	34.64
11-14	29.09	26.77	30.53	34.52	37.34

TABLE A1.5

Phenylpropanolamine Serum concentrations after the administration of 100 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	CONCENTRATION OF PPA IN SERUM (ng/mL)					
	GB	AH	JS	WB	JM	MEAN
0	0	0	0	0	0	0
0.17	68.31	23.36	30.61	8.06	5.07	27.08
0.33	165.29	168.37	157.59	33.68	173.48	139.68
0.50	255.11	300.19	218.94	152.33	316.01	248.52
0.67	309.58	290.59	339.65	160.02	342.16	288.40
1.00	321.99	308.43	363.53	279.98	390.98	332.98
1.50	480.62	277.26	414.73	339.53	414.16	385.26
2.00	356.22	230.00	437.13	317.27	369.01	341.93
2.50	359.62	195.86	394.45	343.80	352.71	329.29
3.00	255.17	195.09	393.91	267.52	303.06	282.95
4.00	247.94	157.03	328.45	243.15	273.05	249.92
6.00	211.54	128.75	304.18	183.28	193.57	204.26
8.00	108.78	86.68	213.75	152.97	135.12	139.46
10.00	76.71	65.61	128.74	110.43	94.62	95.22
12.50	55.48	44.14	69.47	64.82	74.12	61.61
14.00	44.98	35.52	59.95	35.37	48.64	44.89
16.00	37.56	24.79	49.92	7.79	39.64	31.94

TABLE A1.6

The cumulative urinary excretion (Ae) of PPA after the administration of 100 mg PPA.HCℓ dissolved in 200 mL of water.

TIME (HRS)	Ae (mg)				
	GB	AH	JS	WB	JM
0	0	0	0	0	0
0-1	0	9.31	34.46	2.72	4.17
1-3	18.73	31.08	102.10	15.04	19.12
3-5	55.10	44.66	57.74	27.81	33.62
5-7	61.93	55.05	111.18	35.85	48.84
7-9	65.68	66.86	108.08	44.37	55.76
9-11	69.75	78.14	79.60	52.09	61.97
11-14	73.57	83.59	83.18	58.59	67.39

TABLE A1.7

Phenylpropanolamine Serum concentrations of the multiple dose study (25 mg PPA.HCℓ).

TIME	CONCENTRATION OF PPA IN SERUM (ng/mL)				
(HRS)	GB	AH	JS	WB	JM
0	125.97	72.53	130.79	101.73	104.65
0.17	121.14	153.20	125.58	108.81	94.53
0.33	146.01	152.57	140.94	145.44	184.20
0.50	159.79	179.53	158.30	190.03	187.47
0.67	158.96	161.32	170.18	198.67	181.84
1.00	168.63	155.25	179.67	178.46	176.99
1.50	165.09	123.01	178.62	162.83	155.49
2.00	149.81	112.04	164.70	156.15	138.42
2.50	153.44	99.77	176.54	140.93	126.64
3.00	142.56	85.40	163.94	141.00	113.11
4.00	109.52	74.91	156.05	125.36	98.90
6.00	90.11	58.41	115.97	80.13	75.07
8.00	61.49	43.53	83.34	64.16	58.89
10.00	49.77	30.45	59.09	57.29	43.46
12.50	36.81	23.57	52.37	33.90	26.74
14.00	25.63	21.99	43.10	25.85	20.51
16.00	20.32	13.14	27.83	20.48	15.07

TABLE A1.8

The cumulative urinary excretion (Ae) of the multiple dose study (25 mg PPA.HCℓ).

	AMOUNT EXCRETED (mg)				
TIME (HRS)	GB	AH	JS	WB	JM
0	17.10	23.49	9.45	20.76	23.96
0-1	26.26	30.06	24.45	27.34	29.67
1-3	39.07	41.96	44.97	38.72	41.31
3-5	46.36	50.34	53.26	49.20	48.89
5-7	51.44	57.41	61.60	56.73	53.04
7-9	54.70	61.76	66.80	61.26	57.13
9-11	57.55	65.65	70.67	63.95	60.03
11-14	60.20	68.13	73.17	67.72	61.81

APPENDIX 2

TABLE A2.1

Observed and model-predicted serum concentrations of phenylpropanolamine (using model A2) following administration of a 25, 50 and 100 mg solution of the drug (Mean Data).

TIME (HRS)	25 mg DOSE MEAN DATA		50 mg DOSE MEAN DATA		100 mg DOSE MEAN DATA	
	OBS.[1]	PRED.[2]	OBS.	PRED.	OBS.	PRED.
0	0	0	0	0	0	0
0.17	12.39	34.38	35.56	58.08	27.08	99.43
0.33	45.39	56.37	111.03	95.79	139.68	170.86
0.50	84.29	71.96	137.26	123.05	248.52	228.23
0.67	92.65	81.91	130.47	140.41	288.40	270.61
1.00	95.25	91.08	149.23	158.44	332.98	321.74
1.50	95.16	92.06	172.73	162.65	385.26	350.34
2.00	81.13	87.11	151.60	156.19	341.93	348.37
2.50	78.49	80.53	148.12	146.46	329.29	332.67
3.00	69.84	73.81	136.38	136.09	282.95	311.42
4.00	58.25	61.50	112.92	116.53	249.92	265.95
6.00	40.97	42.47	86.66	84.94	204.26	188.46
8.00	31.68	29.32	60.83	61.88	139.46	132.75
10.00	22.33	20.24	53.34	45.08	95.22	93.46
12.50	15.85	12.73	26.40	30.35	61.61	60.27
14.00	12.18	9.64	21.58	23.93	44.89	46.32
16.00	8.31	6.66	15.48	17.44	31.94	30.61

1 Observed serum concentration (ng/mL)

2 Predicted serum concentration (ng/mL)

TABLE A2.2

Observed and model-predicted serum concentrations of phenylpropanolamine (using model B1) following administration of a 25, 50 and 100 mg solution of the drug (Mean Data).

TIME (HRS)	25 mg DOSE MEAN DATA		50 mg DOSE MEAN DATA		100 mg DOSE MEAN DATA	
	OBS.[1]	PRED.[2]	OBS.	PRED.	OBS.	PRED.
0	0	0	0	0	0	0
0.17	12.39	32.99	35.56	57.93	27.08	96.01
0.33	45.39	55.75	111.03	96.24	139.68	168.25
0.50	84.29	72.15	137.26	122.98	248.52	225.77
0.67	92.65	83.20	130.47	140.67	288.40	269.64
1.00	95.25	94.15	149.23	158.57	332.98	326.02
1.50	95.16	94.93	172.73	162.80	385.26	359.11
2.00	81.13	87.91	151.62	156.24	341.93	357.83
2.50	78.49	78.95	148.12	146.38	329.29	340.26
4.00	58.25	56.65	112.92	116.28	249.92	265.30
6.00	40.97	39.92	86.65	84.78	204.26	185.10
8.00	31.68	29.83	60.83	61.85	139.46	132.19
10.00	22.33	22.63	53.34	45.14	95.22	95.79
12.50	15.85	16.08	26.40	30.45	61.61	64.51
14.00	12.18	13.11	21.58	24.05	44.89	50.95
16.00	8.31	9.98	15.48	17.55	31.94	37.21

1 Observed serum concentration (ng/mL)

2 Predicted serum concentration (ng/mL)

APPENDIX 3

TABLE A3.1 Parameter estimates from computer modelling of the 25, 50 and 100 mg solution data.

Model	Parameter	Subjects					Mean
		GB	AM	JS	WB	JM	Data
25 mg solution study							
1BCM	k_{a1}	1.02	2.59	1.51	1.37	3.2	2.08
1k	V_1	293	351	287	232	275	295
linear	k_e	0.175	0.178	0.143	0.220	0.25	0.184
elim.	t_{lag}	0.14	0.08	0.16	0.26	0.19	0.15
	r^2	0.994	0.974	0.979	0.965	0.911	0.974
1BCM	k_0	45401	50677	57658	65655	82833	60267
Zero-	V	330	321	315	298	251	311
order Abs.	K_{10}	0.14	0.15	0.14	0.14	0.249	0.136
linear	T	0.5	0.5	0.5	0.49	0.5	0.49
elim.	r	0.838	0.985	0.87	0.91	0.96	0.97
2BCM	k_{a1}	1.08	1.43	0.85	0.99	1.69	1.13
1K	V_1	286	214	200	200	200	200
linear	k_{12}	0.01	0.57	0.286	0.144	0.48	0.43
elim.	k_{21}	0.16	0.29	0.22	0.26	0.30	0.29
	t_{10}	0.08	0.18	0.45	0.50	0.50	0.42
	r	0.995	0.980	0.987	0.970	0.938	0.978
50 mg solution study							
1BCM	k_{a1}	2.42	4.29	1.17	0.796	3.49	2.02
1 k	V_1	353	353	272	254	317	318
linear	k_e	0.149	0.178	0.161	0.177	0.167	0.158
elim.	t_{lag}	0.08	0.07	0.182	0.18	0.11	0.126
	r	0.983	0.91	0.963	0.983	0.96	0.987
1BCM	k_0	82907	99986	95179	80568	99999	88133
Zero-	V	270	200	290	285	200	264
order Abs.	k_{10}	0.13	0.164	0.13	0.13	0.149	0.12
linear	T	0.5	0.367	0.499	0.5	0.437	0.49
elim.	r	0.97	0.93	0.81	0.75	0.972	0.962
2BCM	k_{a1}	1.78		0.72	0.74	2.51	1.99
1 k	V_1	265	297	200	227	231	281
linear	k_{12}	0.34	0.01	0.195	0.02	0.28	0.01
elim.	k_{21}	1.0	0.01	0.34	0.06	0.71	0.77
	k	0.192	0.169	0.236	0.175	0.208	0.16
	t_{10}	0.103	0.416	0.479	0.115	0.132	0.07
	r	0.983	0.911	0.97	0.981	0.97	0.99

100 mg solution study							
1BCM	k_{a1}	0.142	2.57	1.07	0.761	1.61	1.41
1 k	V_1	258	357	225	231	266	274
linear	k_e	0.205	0.194	0.163	0.202	0.184	0.175
elim.	t_{lag}	0.21	0.08	0.274	0.213	0.20	0.186
	r	0.967	0.95	0.984	0.972	0.965	0.983
1BCM	k_0	155355	137020	160000	146250	171259	158240
Zero-	V	203	224	200	245	211	231
order Abs.	k_{10}	0.14	0.157	0.14	0.14	0.14	0.11
linear	T	0.5	0.49	0.5	0.5	0.49	0.49
elim.	r	0.893	0.976	0.821	0.73	0.931	0.924
2BCM	k_{a1}	0.886	1.36	1.05	0.581	0.989	0.918
1 k	V_1	200	213	203	200	200	200
linear	k_{12}	0.167	0.525	0.01	0.06	0.26	0.22
elim.	k_{21}	0.34	0.48	0.01	0.29	0.45	0.55
	k	0.279	0.299	0.15	0.25	0.26	0.25
	t_{10}	0.49	0.188	0.19	0.46	0.46	0.35
	r	0.98	0.962	0.983	0.968	0.98	0.985

[1] Correlation coefficient as an indication of the goodness of fit

APPENDIX 4

TABLE A4.1 Urine pH Readings.

SUBJECT		DOSE			SUBJECT		DOSE		
	TIME (HRS)	25	50	100		TIME (HRS)	25	50	100
AH	0	5.16	5.59	5.05	GB	0	5.78	5.93	5.25
	0-1	6.63	5.98	5.67		0-1	6.41	6.69	-
	1-3	7.40	6.00	7.35		1-3	6.94	6.92	6.57
	3-5	7.01	5.51	6.37		3-5	6.56	6.89	6.78
	5-7	5.89	5.44	6.72		5-7	6.77	6.17	5.88
	7-9	6.28	5.56	6.82		7-9	5.91	5.95	6.08
	9-11	5.92	5.26	5.70		9-11	5.55	5.69	5.85
	11-14	5.66	5.47	5.79		11-14	6.21	6.65	5.71
	MEAN	6.24	5.60	6.18		MEAN	6.26	6.36	6.01
	± SD	0.738	0.259	0.75		± SD	0.49	0.48	0.51
WB	0	5.64	5.67	5.58	JM	0	5.37	5.73	5.26
	0-1	7.24	6.96	7.22		0-1	5.37	5.38	7.46
	1-3	7.38	7.50	7.61		1-3	6.06	6.65	6.72
	3-5	6.25	6.97	7.03		3-5	6.36	6.40	6.98
	5-7	6.15	7.21	6.03		5-7	5.28	6.34	6.82
	7-9	6.39	6.95	6.25		7-9	5.54	5.92	6.93
	9-11	5.41	5.94	5.46		9-11	5.92	5.32	6.35
	11-14	5.68	6.09	5.86		11-14	5.70	5.73	5.48
	MEAN	6.26	6.66	6.38		MEAN	5.70	5.93	6.50
	± SD	0.725	0.666	0.80		± SD	0.38	0.48	0.76
JS	0	5.41	5.61	5.90					
	0-1	5.84	6.47	5.78					
	1-3	6.10	7.13	6.52					
	3-5	6.00	7.24	7.21					
	5-7	5.47	6.54	6.62					
	7-9	5.45	5.81	6.09					
	9-11	5.06	5.44	5.38					
	11-14	5.34	6.00	5.43					
	MEAN	5.58	6.28	6.11					
	± SD	0.358	0.67	0.63					

TABLE A4.2 Blood Pressure Readings.

TIME (HRS)	BLOOD PRESSURE				
	SUBJECT				
	AH	GB	WB	JS	JM
1. 25 mg DOSE PPA.HCl.					
0	125/85	115/70	115/80	120/80	130/70
0.666	110/80	110/80	110/65	120/75	120/60
1.5	110/65	110/70	130/90	130/70	120/79
2.5	110/70	110/70	120/80	100/70	100/50
8	100/70	110/70	110/60	110/70	120/60
16	115/75	110/70	115/70	110/70	120/70
2. 50 mg DOSE PPA.HCl.					
0	115/90	115/70	110/90	110/60	120/80
0.666	90/70	120/86	120/90	138/100	130/80
1.5	90/70	130/80	135/85	150/100	130/80
2.5	110/70	150/100	120/76	140/80	150/90
8	110/60	120/70	120/78	110/60	130/70
16	115/90	120/70	120/80	108/70	120/80
3. 100 mg DOSE PPA.HCl.					
0	110/85	105/80	120/80	110/80	135/75
0.666	120/80	120/86	130/85	140/90	170/100
1.5	155/100	136/100	130/80	140/90	170/90
2.5	110/90	118/65	120/65	140/85	130/76
8	120/80	110/80	130/86	120/85	115/75
16	120/80	110/80	120/80	110/80	120/75

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