HPLC ANALYSIS AND PHARMACOKINETICS OF CYCLIZINE

by

Roderick Bryan Walker

A Thesis Submitted to
Rhodes University
in Fulfilment of the
Requirements for the Degree
of

DOCTOR OF PHILOSOPHY

January 1995

School of Pharmaceutical Sciences
Rhodes University
Grahamstown

STUDY OBJECTIVES

Introduction

Cyclizine, is a piperazine derivative that exhibits antihistaminic activity. It is particularly useful for the prevention and treatment of nausea and vomiting associated with motion sickness. Cyclizine is available as an intramuscular injection, tablet, syrup and suppository for both adult and paediatric use. In spite of widespread use of cyclizine as an anti-emetic, there is a paucity of information regarding the pharmacokinetics of the compound, which can probably be accounted for by the lack of a suitable, sensitive analytical method that is necessary to measure the low concentrations of cyclizine likely to be found after administration.

Objectives

The objectives of this study were therefore:-

1. To develop and validate a suitable High-Performance Liquid Chromatographic (HPLC) method with the necessary sensitivity to accurately and precisely quantitate cyclizine and its major metabolite, norcyclizine in serum and urine.

2. To characterize the pharmacokinetics of cyclizine by studying the absorption and disposition of the drug in healthy human volunteers.

3. To evaluate, using both compartmental and non-compartmental pharmacokinetic analyses the data obtained following administration of cyclizine as single doses (intravenous and oral) and following fixed multiple dosing (oral) in man.

ABSTRACT

The investigations detailed in this dissertation have been conducted to address the paucity of pharmacokinetic information, in published literature, pertaining to cyclizine. The areas of investigation have included the selective quantitation of both cyclizine and its demethylated metabolite, norcyclizine in serum and urine, assessment of stability of both compounds in stored biological samples, dosage form analysis, dissolution rate testing of tablets, and bioavailability and pharmacokinetics following administration of an intravenous solution, and tablets to humans.

High-performance liquid chromatography (HPLC) was used as the main analytical technique throughout these studies. An original HPLC method employing ultraviolet detection with a limit of quantitation of 5$\mu g/\ell$ was developed for the determination of cyclizine in serum and both cyclizine and norcyclizine in urine. Solid-phase extraction using extraction columns packed with reversed-phase C_{18} material, and followed by a simple phase-separation step proved successful for the accurate and precise isolation of the compounds. The validated method was applied to the analysis of serum and urine samples from a pilot study in which a single volunteer was administered 50mg of cyclizine hydrochloride. Several samples collected during the pilot study revealed the presence of both drug and metabolite in concentrations below the limit of detection. In order to improve the selectivity and sensitivity of the analytical method an HPLC method with electrochemical detection operating in the "oxidative-screen" mode was developed. The solid-phase extraction procedure was modified slightly and the method found to be precise, accurate, selective and highly sensitive with a limit of quantitation of 1$\mu g/\ell$ for both cyclizine and norcyclizine in both serum and urine. This method was applied to the determination of both compounds after intravenous and oral administration of cyclizine to humans.

HPLC with electrochemical detection was used for the analysis of samples collected during dissolution studies on the batch of tablets used for pharmacokinetic studies. In addition, this method was used to assess content uniformity of the tablets and of samples from the batch of intravenous ampoules of cyclizine lactate. Dissolution studies showed that all tablets tested passed the compendial specifications for cyclizine. Content uniformity assessment revealed that within-batch uniformity existed for both the tablets and ampoules and, therefore, variations in pharmacokinetic parameters for the drug would more than likely be as a result of inter- and intra-individual variability within the subject population.

Pharmacokinetic information for cyclizine was obtained following administration of an intravenous bolus dose of cyclizine lactate as a solution, oral administration of cyclizine hydrochloride as a single dose of 50mg and as fixed multiple doses of 50mg every 8 hours for five days. Further information was acquired following administration of single doses of 100mg and 150mg cyclizine hydrochloride.

Data collected from these studies were evaluated using both compartmental and non-compartmental techniques. Cyclizine was rapidly absorbed following oral administration with a mean k_a = 1.54 hr^{-1} and was found to have an absolute bioavailability (F) of 0.47. The presence of norcyclizine in serum following oral and not intravenous dosing suggests cyclizine is susceptible to "first-pass" metabolism in either the gut wall or the liver. Mean Cl_{TOT} determined following the intravenous dose was 0.865 ℓ/hr/kg. The mean Cl_{TOT} of 0.823 ℓ/hr/kg calculated following oral dosing, using a unique value of F for each subject compared favourably with that obtained following intravenous dosing. Renal clearance of cyclizine is negligible indicating that non-renal routes of elimination account for the majority of removal of cyclizine form the body. Cyclizine is extensively distributed and the mean V_Z following an intravenous dose was 16.70 ℓ/kg. This value is lower than that calculated from all oral studies from which the mean V_Z was determined to be 25.74 ℓ/kg. Cyclizine is eliminated slowly with a mean elimination t½ = 20.11 hours. Cyclizine dose not appear to follow dose-dependent kinetics and therefore, inability to predict steady state levels are more than likely due to accumulation as a result of frequent dosing rather than saturation of elimination mechanisms.

Modelling of intravenous data to one-compartment (1BCM), two-compartment (2BCM) and three-compartment models indicated that the pharmacokinetics of cyclizine can be adequately described by a 3BCM. The drug is rapidly distributed into a "shallow" peripheral compartment (α = 9.44 hr^{-1}, and k_{21} = 2.09 hr^{-1}), and slowly distributed to the "deep" peripheral compartment (β = 0.451 hr^{-1}, and k_{31} = 0.120 hr^{-1}). Modelling of all oral data indicated that a 2BCM best described the pharmacokinetics of the drug, however, distribution to the peripheral compartment is not as rapid as to the "shallow" peripheral compartment following the intravenous dose. Mean distribution parameters were α = 0.64 hr^{-1} and, k_{21} = 0.39 hr^{-1}. Mean Cl_{TOT} following intravenous dosing of 0.70 ℓ/hr/kg was similar to the mean Cl_{TOT} of 0.73 ℓ/hr/kg determined after oral dosing. The mean distribution volume at steady state determined following intravenous dosing (17.78 ℓ/kg) was lower than that obtained from the oral studies (25.52 ℓ/kg). The mean terminal elimination half-lives calculated for cyclizine following fitting of intravenous and oral data was 25.09 hours.

In general, mean pharmacokinetic parameters calculated following fitting of data to a 2BCM after oral administration correlate closely with those calculated using non-compartmental techniques. However, the pharmacokinetics following intravenous dosing are better described by a 3BCM and a close correlation between parameters estimated using noncompartmental techniques and compartmental techniques is evident when a 3BCM model is used.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the following people:-

My supervisor, Professor I. Kanfer for his patience, guidance, understanding and assistance throughout the course of my studies. Also, for providing superb laboratory facilities, financial support and the opportunity to attend the Eleventh International Symposium on Column Liquid Chromatography in Amsterdam and the Pharmacy World Congress '91 in Washington, DC.

All my colleagues in the laboratory for their congenial company, support and numerous forms of assistance. Specific thanks to Mike Skinner, Chris Stubbs and Neil Sparrow for their assistance in the beginning. Also my colleagues, Roslind Dowse and Eric Smith in the Division of Pharmaceutics for their encouragement and support. Beverley Glass for her help in preparation of molecular structures and Susan Abrahams for her help in preparing chromatograms for this dissertation. Carol Hubbard for her patience and help with the printing of this thesis.

Andy Soper and Owen Campbell for maintaining and repairing equipment. To Sagaran Abboo for his help in the laboratory and during bioavailability trials and also Leon Purdon for his various forms of assistance. Special thanks also go to Dr Ann Kench, and Sisters Penny Bernard and Penny Jaffray for their invaluable help during bioavailability trials.

The Head, Professor H. Parolis, the Dean, Dr B.J. Wilson and the staff of the School of Pharmaceutical Sciences for their assistance, advice and use of departmental facilities. Also for affording me the opportunity to gain invaluable teaching experience during this time.

The Council for Scientific and Industrial Research, Foundation for Pharmaceutical Education and Millipore (SA) for their generous financial assistance.

My parents and family for their continued support, encouragement and assistance throughout my studies.

My wife, Rosy, for her love, patience, understanding, encouragement and help during the preparation of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 PHYSICOCHEMICAL PROPERTIES OF CYCLIZINE

1.1.1 Description

Cyclizine is 1-(diphenylmethyl)-4-methylpiperazine or 1-benzhydryl-4-methylpiperazine (1,2).

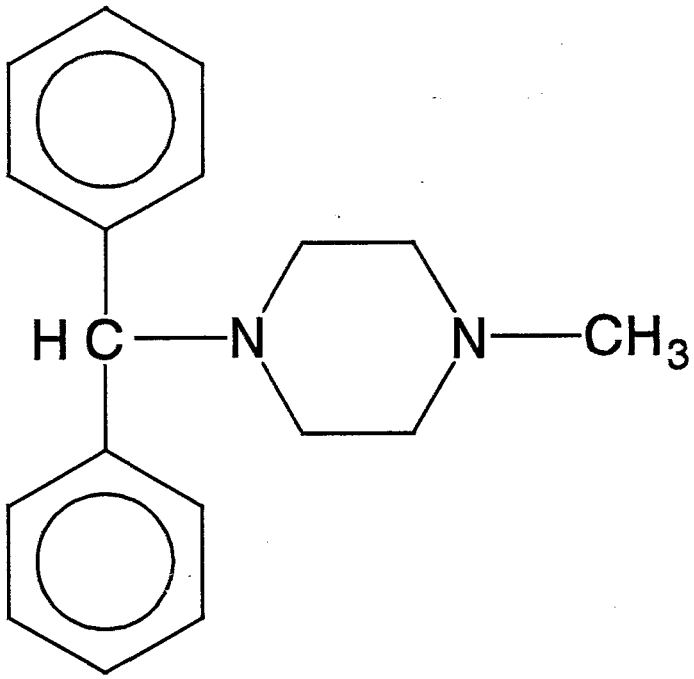


Figure 1.1 Cyclizine base (molecular mass (MM)=266.40)

Cyclizine base is a white or creamy white, almost odourless crystalline powder (1,2,3).

The clinically used form is cyclizine hydrochloride or 1-(diphenylmethyl)-4-methylpiperazine monohydrochloride (MM=302.85). It is a white, almost odourless crystalline powder or colourless crystals with a bitter taste (2,3,4,5).

Cyclizine lactate (MM=356.46) is a solution of cyclizine prepared with the aid of lactic acid, and is used parenterally (2,4).

1.1.2 Synthesis

Baltzy *et al* (6) in 1949, described a generalized synthesis for the benzhydrylmethylpiperazines, in which the required carbinols were prepared from the appropriate aldehydes and Grignard reagents.

The preparation of cyclizine is shown in Figures 1.2 and 1.3. Initially a monalkyl intermediate of piperazine (A) is produced. This is achieved by the introduction of a carbethoxy substituent on one nitrogen atom (B), utilizing ethyl chloroformate in a buffered solution, then alkylating the second nitrogen atom (C) and finally removing the carbethoxy group by hydrolysis (D) (7).

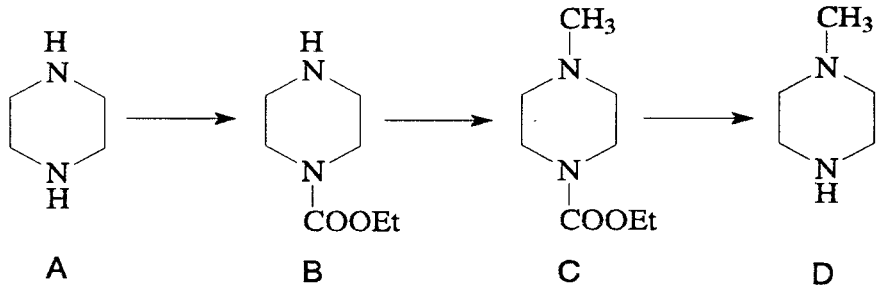


Figure 1.2 Preparation of N-methylpiperazine.

The N-methylpiperazine (D) is then condensed with benzhydryl chloride, prepared from diphenylcarbinol by Grignard synthesis, resulting in the formation of cyclizine (1,7).

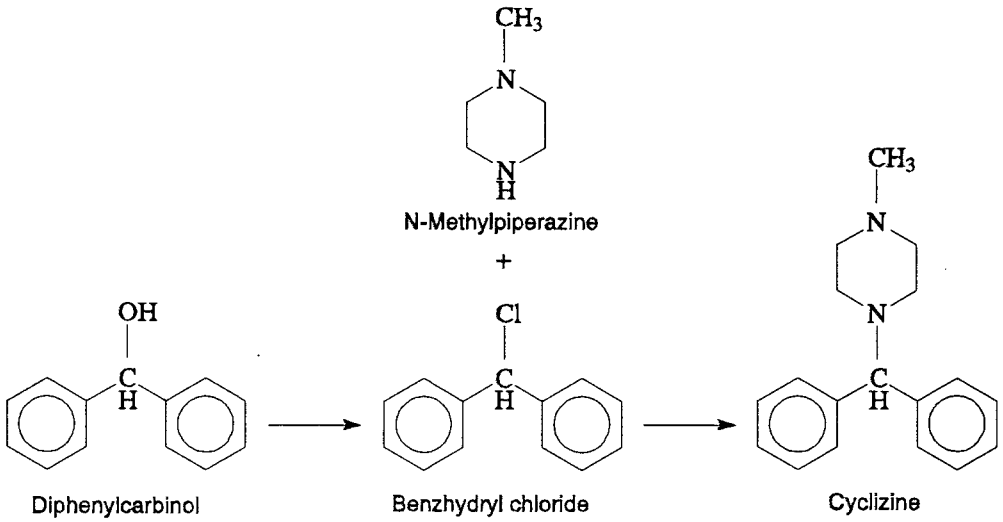


Figure 1.3 Preparation of cyclizine.

1.1.3 Melting Range

Cyclizine hydrochloride has a melting point of 285°C with decomposition whereas cyclizine as the free base has a melting range of 106°C to 109°C (2,4).

1.1.4 Solubility

Tables 1.1 and 1.2 list the solubilities of cyclizine as both the free base and the monohydrochloride salt at 20°C (2,3).

Table 1.1 Solubility of Cyclizine.

SOLVENT	SOLUBILITY
Water	Insoluble
Alcohol	1 in 6
Ether	1 in 6
Chloroform	1 in 0.9

Table 1.2 Solubility of Cyclizine Hydrochloride.

SOLVENT	SOLUBILITY
Water	1 in 115
Alcohol	1 in 115
Ether	1 in 75
Chloroform	Insoluble

1.1.5 Dissociation Constant

The ionization constants of cyclizine, a basic drug, in a 50% methanolic solution have been reported as $pK_1=2.54$ and $pK_2=7.92$ (6). Newton *et al* (8) obtained $pK_1=2.16$ and $pK_2=8.05$ in aqueous solution at 24.5 °C. Cyclizine monohydrochloride has a $pK_a=8.2$ at 20 °C (5,9). The evidence of two ionization constants is probably due to the availability of two nitrogen atoms in the piperazine ring. The disparity of these values which would be expected to be equivalent is most likely a result of difficulty in the protonation of the second nitrogen atom due to electrostatic effects when the piperazine ring is in the chair configuration.

1.1.6 pH

The pH of a saturated solution of cyclizine as the free base in carbon dioxide-free water is between 7.6 and 8.6. A 2% solution of cyclizine hydrochloride has a pH between 4.5 and 5.5, determined potentiometrically in an alcohol (2 volume) and water (3 volume) mixture (5). Cyclizine lactate solution for injection is prepared *in situ* and has a pH of between 3.2 and 4.7 (2,3,4).

1.1.7 Crystal Structure

The crystal structure was determined (10) using single crystals of cyclizine hydrochloride, purified by recrystallization from ethanol. Intensity data were collected on an automatic four-circle diffractometer with monochromated Mo K_α radiation. Cyclizine hydrochloride lies on a crystallographic plane of symmetry. The crystal structure is held together by van der Waal's packing of ion pairs linked by hydrogen bonds.

The distance between the nitrogen atom and the centroid of the phenyl ring is 6.03Å. This is close to that of other compounds possessing antihistaminic activity, despite differences in the respective chemical classes of the drugs. The cell dimensions are a=11.833Å, b=13.631Å, c=10.023Å and the final discrepancy factors, R and R_w, were 0.047 and 0.041 respectively.

1.1.8 Ultraviolet Absorption Spectrum

The ultraviolet absorption spectrum of cyclizine hydrochloride in a mixture of 0.05M phosphate buffer (pH=3) and acetonitrile (70/30) is depicted in Figure 1.4. The spectrum was obtained using an HP 1040A diode array detector (Hewlett Packard, Palo Alto, CA, U.S.A.).

Despite the differences in the solvent used, the absorption spectrum of cyclizine hydrochloride is similar to that depicted by Benezra (1) and other workers (11).

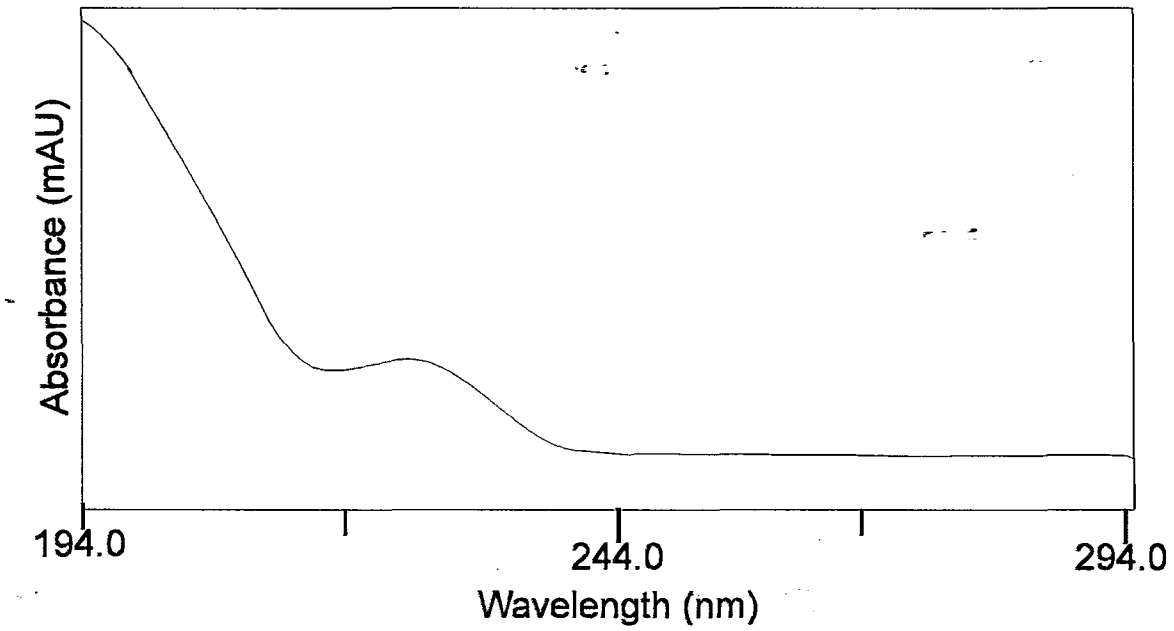


Figure 1.4 Ultraviolet absorption spectrum of cyclizine hydrochloride showing λ_{MAX} values at 230nm and 200nm.

1.2 PHARMACOLOGICAL PROPERTIES OF CYCLIZINE HYDROCHLORIDE

1.2.1 Structure Activity Relationships

In general, agonists and their competitive antagonists are assumed to act on common receptors and therefore show structural similarities. This, however, is not true for compounds such as the antihistamines (12). Indeed, there is a clear chemical and stereochemical correlation amongst the antihistaminics themselves despite the fact in spite of the fact that they belong to five chemically distinctive classes (i.e. ethylenediamine, ethanolamine, alkylamine, piperazine and phenothiazine) (10,13,14).

The pharmacodynamic entity of histamine and its antagonists, is a substituted ethylamine moiety (13,14). Most of the histamine-1 (H_1) receptor blockers have a tertiary amino group linked by one, two or three carbon atoms, to two aromatic substituents (15). This amino group reacts with the anionic site on the H_1-receptor (16).

An interesting phenomenon is that the ethylamine grouping which appears to be essential for activity of the most commonly used antihistamines is also shared by a number of the naturally occurring amines, including acetylcholine, 5-hydroxytryptamine, epinephrine and histamine itself (14). As a result of this structural similarity to some of the biogenic amines, most antihistaminics will cross-react at these receptor sites. Thus, most antihistaminics will elicit weak anti-adrenergic, anticholinergic and antiserotonergic effects (17). This may be an indication of how some antihistamines are able to prevent nausea, vomiting and/or motion sickness by their action on muscarinic receptors of the cholinergic nervous system.

Reports in the literature suggest that the presence of two aromatic rings is useful for the enhancement of antihistaminic activity and that the strongest antagonism occurs when at least one ring is able to assume a fixed distance from the amino nitrogen atom (18). Witiak (16) suggests that antihistamines probably do not interact with the entire receptor area to which histamine binds, but that they rather interact with regions adjacent to the receptor site. These interactions appear necessary for the affinity of the antagonist for the histamine receptor.

In the case of cyclizine hydrochloride, the nitrogen in the ethylamine moiety is incorporated as part of a piperazine ring. The presence of the piperazine ring may prolong the onset of action and enhance the duration of activity of the drug molecule (16,19).

1.2.2 Mode of Action of Cyclizine Hydrochloride

Antihistaminic drugs, or more specifically, H_1-receptor antagonists are able to completely antagonise the effects of histamine at the H_1-receptor. These compounds do not combine chemically with histamine to cause inactivation, nor do they interfere with histamine release from storage sites (10,13). Essentially it is important to remember that these compounds are not able to reverse the effects of histamine once they have occurred (17).

Marshall (20) determined cyclizine to be a competitive antagonist of histamine by use of the relationship between pK_a and pA_2 values. The mode of action of antihistamines was thought to be via a simple reversible competitive antagonism of histamine at the H_1-receptor site (14). More recently (15), the antihistamines are thought to act by occupancy of receptors on the effector cell, to the exclusion of agonist molecules, without themselves initiating a response.

There are five chemically distinctive groups of antihistaminic drugs of which the piperazines are best known for their ability to antagonize motion sickness (13). Cyclizine hydrochloride falls into this

particular class of antihistaminic and its action as an anti-emetic is probably more readily accounted for by the marked anticholinergic action rather than the antihistaminic activity it may possess (14,21).

Motion sickness occurs as a result of excessive vestibular stimulation or as a result of the difference between sensory information from the labyrinths and that from other sensory inputs regarding the orientation of the body. Vertigo, nausea, and vomiting occurs in Mènèire's disease as a result of spontaneous bursts of activity which occur in the semicircular canals and in the sensory nerves conducting impulses from them. In both instances, antihistamines and anticholinergics are the drugs of choice (9,21). The mechanism by which these symptoms may be blocked by antihistamines is graphically depicted in Figure 1.5.

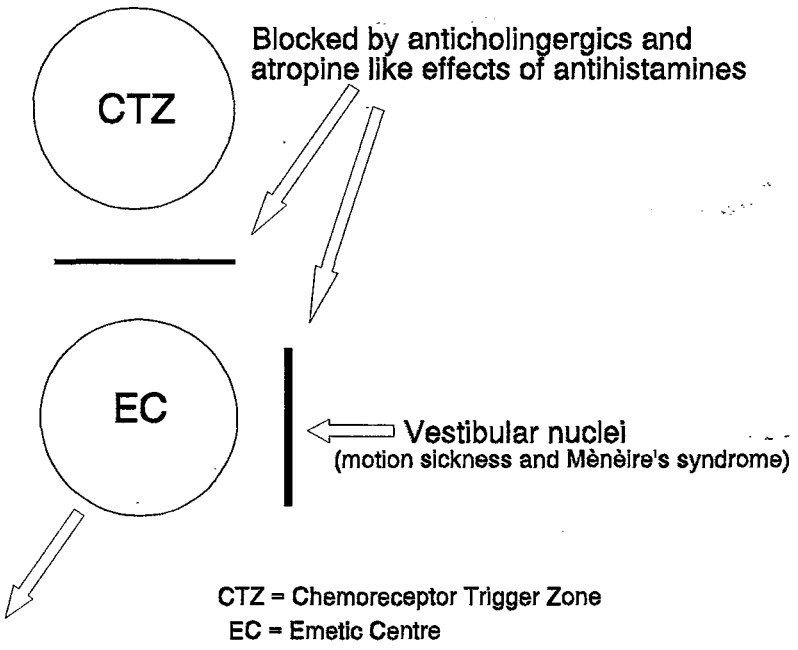


Figure 1.5 Blockade of stimuli evoking central vomiting.

1.2.3 Side Effects and Uses of Cyclizine Hydrochloride

1.2.3.1 Pharmacological Action

In addition to directly antagonizing histamine at the receptor level, the antihistaminics have several other pharmacological actions which may or may not be considered as side effects when selecting the drug solely for its pharmacological action. In this respect, cyclizine is no exception.

One of the more prevalent side effects of the antihistamine type compounds is that of sedation. Cyclizine hydrochloride was found to cause sedation forty minutes after administration of the normal adult dose (50mg) in 68% of subjects and in 52% of subjects to whom half the normal adult dose was administered. After six hours the sedation was still evident in a large percentage of the subjects (22). In another study, cyclizine was found to exhibit 28% more sedation than the placebo employed in the study (23). Nicholl *et al* (24) have reported that cyclizine had the opposite effect and caused restlessness. This effect was also observed by Clarke and Dundee (22) who noted that fewer than 10% of subjects exhibited restlessness when given the normal adult dose.

Cyclizine was shown to increase the sleeping time of rats that had been injected with pentobarbital. This may be due, in part, to the ability of some of the N-benzhydryl piperazines to cause hypothermia, which is known to be responsible for the enhancement of sleeping time with barbiturates (25).

Antihistamines, are known to be multipotent antagonists possessing antihistaminic, anticholinergic and antiserotinergic activity (26). Several studies have highlighted some of the parasympatholytic effects of cyclizine, including dryness of mouth, dysuria, headaches and palpitations (14). In a study, conducted by Chinn *et al* (23), side effects such as dizziness, dryness of mouth, blurred vision, sweating, headaches and excessive fatigue after cyclizine administration were noted and expressed as a percentage of subjects participating in the study. The side effects were in most cases, present in the experimental group and not the placebo group. Clarke and Dundee (22) reported a degree of dryness of mouth and an incidence of dizziness of between 20-35 percent in subjects dependent on the dose administered.

Cyclizine hydrochloride has been reported as having an effect on the cardiovascular system. The drug was found to decrease the pulse rate in both supine and standing human subjects. This decrease was significantly less than that of the placebo employed (22).

Norton *et al* (27) have shown that doses of cyclizine higher than 1mg/kg cause a decrease in blood pressure in anaesthetized cats and dogs. They also ascertained that the fall in blood pressure noted on stimulation of the preganglionic (cervical) fibres of the vagus was blocked by cyclizine. In addition, there was no effect on blood pressure response to injected acetylcholine, yet low doses of cyclizine (0.5mg/kg) blocked falls in blood pressure produced by histamine injection for several hours. Cyclizine (8mg/kg) completely blocked the fall in blood pressure due to serotonin injection in cats (27).

Cyclizine is often administered to counteract the emetic effects of narcotic analgesics given during myocardial infarction or left ventricular failure. Intravenous cyclizine was found to decrease or prevent falls in blood pressure (28).

Tan *et al* (29) however, reported that cyclizine therapy in conjunction with diamorphine was inappropriate as cyclizine significantly increased heart rate as well as right atrial, pulmonary arterial, systemic arterial, left and right ventricular filling pressures. In addition they found that cyclizine negated the beneficial veno-dilatory effect of diamorphine.

These effects are consistent with the findings of Gott (30), who noted in two cases of intentional abuse of cyclizine, that the subjects presented with tachycardia and hypertension amongst other side effects. Occasional tachycardia in patients treated with cyclizine to prevent post operative nausea and vomiting (31) and mild hypertension have also been reported (27).

Hallucinations, euphoria and dilated pupils were prevalent in cases where cyclizine was taken in doses far exceeding the recommended dosage (30). A further report of hallucinations after larger than recommended doses of intravenous cyclizine has been published (32). A significant increase in reaction time and subjective effects (ie. drowsiness, lethargy, dreaminess) were noticed in subjects that received cyclizine hydrochloride (100mg) when compared to those receiving cyclizine hydrochloride (50mg) or a lactose placebo (33).

Cyclizine tends to decrease spontaneous motor activity at a low dosage (5mg/kg) level, yet in higher doses (20mg/kg) causes an increase in such activity. It has no effect on conditioned response even in toxic doses, but produced pilo-erection and tremors at these levels (25). Subcutaneous administration of cyclizine hydrochloride in saline (1mg/kg and 10mg/kg) to male mice significantly enhanced locomotor activity of the animals and potentiated opioid-induced hyperactivity but had no effect on amphetamine-induced locomotor stimulation (34). This suggest that H_1-receptor antagonist effects may be related with opioid mechanisms.

In isolated organ studies using guinea pig and rabbit ileum, cyclizine was found to be weakly effective in the reduction of acetylcholine induced spasm (25,27). In the ileum of anaesthetized cats, cyclizine was found to relax the tone and motility at levels below that necessary to decrease blood pressure (27).

Cyclizine is known to increase the lower oesophageal sphincter pressure in man to a pressure above that of 13cm/H_2O which is the pressure at which reflux occurs (35). The mean increase in this barrier pressure induced by cyclizine was reported as 14.2cm/H_2O and hence cyclizine reduces the likelihood of gastro-oesophageal reflux during anaesthesia (36).

Reports show that cyclizine has no adrenolytic activity, but does exhibit some fungistatic activity. Also, normal doses of the drug do not appear to induce mydriasis to any degree (27).

Cyclizine has been tested for antihistaminic activity using the "flare and wheal" method. It was found to have a comparatively potent antihistamine action when compared with an analogue, chlorcyclizine but the duration of the response was short (37). When compared to triprolidine, using the "flare and wheal" method cyclizine was considered a weak antihistamine (33). Confirmation of this antihistaminic activity was shown by the reduction in mortality rate of guinea pigs exposed to nebulized histamine. Cyclizine also exhibited a mild local anaesthetic action similar to that of procaine, when compared using the guinea pig wheal method (27).

Pain at the intramuscular injection site after cyclizine administration has been reported as mild and transitory (38). In another study, pain was reported in approximately 15-30 percent of subjects under investigation (22). The cause of pain is probably a result of injection of the solution of cyclizine lactate whose pH is between 3.2 and 4.7.

1.2.3.2 **Uses of Cyclizine**

The use of cyclizine hydrochloride for the prevention and treatment of motion sickness, whether administered orally, intramuscularly or intravenously are well documented (9,13,14,23,27,37,39). Other clinical uses include the treatment of symptoms produced by Méneire's disease, other labyrinthine disturbances and other types of vertigo (9,15,27).

In some instances cyclizine has been administered to prevent vomiting induced by some narcotic analgesics (for example: morphine) (28,32). Cyclizine has been described as the drug of choice for the prevention of emetic sequelae due to the partial opioid antagonist meptazinol (40). Cyclizine has recently found use for the prevention of post operative nausea and vomiting when administered as a pre-medication or just prior to the end of surgery (22,24,27,31,36). It has also found some use in the prevention of irradiation sickness and drug induced vomiting (2).

1.2.4 Contra-indications

Cyclizine hydrochloride has on several occasions been implicated in foetal abnormalities in experimental animals, when administered in early gestation (9,13,14,15). To date, no teratogenic effects have been reported in humans. Cyclizine is however contra-indicated in pregnancy.

Due to the anticholinergic effects of cyclizine it should be used with extreme caution in conditions which may be exacerbated by atropine, such as glaucoma and prostatic hypertrophy (41).

Cyclizine should be used with caution when administered post-operatively due to it hypotensive action (2).

1.2.5 Drug Interactions

Cyclizine should not be used prior to methohexitone anaesthesia because of the possibility of increased tremor and muscle movements (27).

The sedative action of central nervous system depressants including alcohol, barbiturates, hypnotics, narcotic analgesics, sedatives and tranquilizers may be potentiated by cyclizine (15,29), and the side effects of anticholinergic drugs such as the tricyclic antidepressants may be enhanced by concomitant administration of the drug (15,29,41).

Cyclizine has been shown to be incompatible with pethidine in solution (27), and with oxytetracycline hydrochloride, chlortetracycline hydrochloride and penicillin injections (2).

1.3 PHARMACOKINETICS OF H_1-RECEPTOR ANTAGONISTS - SPECIFICALLY THE PIPERAZINE COMPOUNDS INCLUDING CYCLIZINE HYDROCHLORIDE

In spite of the widespread use of these agents over an extended period there is a paucity of information regarding the pharmacokinetics of these compounds in humans. The dearth of information regarding such pharmacokinetic data may be attributed to (42):-

i) the fact that the compounds have been in clinical use for several years prior to any interest or emphasis on pharmacokinetics, or,

ii) the fact that the antihistamines are potent drugs and require only small amounts to be present to elicit an effect and are therefore present in low concentrations in body fluids necessitating highly sensitive, selective assay methods to measure these, and such techniques have only recently been developed, or,

iii) the fact that routine blood concentration monitoring is unnecessary due to the "minor" side-effects of these compounds.

The antihistamines of the benzhydrylpiperazine series of drugs are best known for their ability to prevent and treat motion sickness and have been extensively used with little knowledge regarding their physiological distribution, metabolism and elimination (43). Chlorcyclizine, the oldest of the group, is an H_1-antagonist with a prolonged action (44) and is no longer available. Cyclizine is best known for use as an anti-emetic. Meclizine is purported to have potent anti-emetic activity with a long (24hr) duration of action (2). Buclizine is less well known than either cyclizine or meclizine and hydroxyzine has seen more use as an anxiolytic, with a duration of action of between 4 to 6 hours (2), rather than as an antihistamine (13). Cinnarizine has a quick onset and short duration of action and possesses the properties and uses of the antihistaminics, but is used for the symptomatic treatment of nausea and vertigo due to Mènière's disease (2).

1.3.1 Absorption

In general H_1-receptor blockers appear to be well absorbed from the gastro-intestinal tract, effects developing within 30 minutes (15) and peak plasma concentrations being reached one to three hours after dosing (45). Rapid absorption of cyclizine has been confirmed by Griffin and Baselt (46) who showed, following a single 50mg oral dose to one volunteer, a time to peak (t_{max}) concentration (69ng/ml) of two hours, with the subjective sedative effects noted at two hours. In another study the t_{max} concentration (49ng/ml) was slightly delayed and occurred at three hours following a dose of 50mg cyclizine hydrochloride to a single volunteer (47). The reported concentration of 80μg/ml (48) is likely to have occurred as a result of massive oral ingestion of cyclizine.

Other drugs of the benhydrylpiperazine series have been subjected to pharmacokinetic studies and the results of which give credence to the fact that these compounds are rapidly and completely absorbed. The results from three independent studies on cinnarizine are depicted in Table 1.3.

Table 1.3 Absorption and Elimination Parameters of Cinnarizine.

Formulation	*C_{max} (ng/ml)	**t_{max} (hr)	***$t_{½}$ (hr)	Reference
Capsule A 75mg (N=6)	122 ± 75	3.3 ± 1.6	2.8 ± 1.0	Puttemans *et al* (49)
Capsule B 75mg (N=6)	110 ± 133	4.1 ± 2.0	3.2 ± 2.1	
Capsule 75mg (N=12)	230 ± 130	2.6 ± 1.0	3.4 ± 0.8	Morrison *et al* (50)
Tablet 75mg (N=12)	160 ± 130	3.4 ± 1.2	3.0 ± 1.5	
Tablet A 50mg (N=6)	76 ± 35	2.3 ± 0.4	3.0 ± 1.5	Hundt *et al* (51)
Tablet B 50mg (N=6)	89 ± 42	2.4 ± 1.1	5.3 ± 1.6	

* Peak Serum Concentration
** Time to Reach Peak Serum Concentration
*** Elimination Half-Life

Cinnarizine appears to be well absorbed and the time to reach peak concentration is in agreement with that reported by Paton and Webster (42) of between one and three hours for the H_1-receptor antagonists. The obvious disparity in peak concentration values may be explained by dosage form differences.

Fouda *et al* (52) studied the pharmacokinetics of hydroxyzine hydrochloride in four male subjects. The results of the study revealed that hydroxyzine hydrochloride was consistently absorbed giving peak serum concentrations between two and four hours after oral administration. The mean absorption half-life of hydroxyzine was found to be 1.02 hours, indicating extremely rapid absorption.

In studies to correlate pharmacokinetic and pharmacodynamic effects of hydroxyzine hydrochloride in human subjects, Simons *et al* (53,54,55,56) reported that irrespective of age or disease state the time to reach peak serum concentrations was approximately 2 hours. There were differences in the peak concentrations measured depending on age or disease state.

Meclizine hydrochloride when administered to a male volunteer (25mg) appeared to be rapidly absorbed with a peak plasma level of 80ng/ml at 4 hours (57). A bioavailablity study using beagle dogs confirmed that absorption of meclizine dihydrochloride is rapid and a peak concentration of 211ng/ml was observed 2 hours after the dose (58). Meclizine dihydrochloride was found to be better absorbed intranasally than orally (59). The maximum plasma concentrations were similar within species, but the time to reach peak concentrations were at least six fold faster for intranasal delivery.

1.3.1.1 **Bioavailability**

Absolute bioavailability of most antihistaminic compounds is not known as there are only a few intravenous preparations available for comparative use (42,45). Land and Bye (60) did not perform a bioavailability study when they administered 50mg cyclizine hydrochloride intravenously to a single male volunteer. The peak concentration was 350ng/ml observed within a few minutes of administration.

The absolute bioavailability of cinnarizine is not possible to determine as there are no intravenous preparations of cinnarizine. Investigations into the improvement of the bioavailability of the drug have been undertaken by Tokumura *et al* (61,62). The evaluation involved administration of cinnarizine ß-cyclodextrin complex with another compound which competes with the molecule for complex formation. In this instance oral bioavailability, assessed by area under the plasma concentration versus time curve (AUC) and peak concentrations were found to be 1.9-2.7 fold that of either the inclusion complex or cinnarizine when administered alone. In addition, the oral bioavailability of the drug from an oleic acid solution in an hard gelatin capsule was found to be greatly enhanced (i.e. peak concentration and AUC were 2.9 fold and 4 fold those after tablet administration).

1.3.2 **Distribution**

In 1965 Kuntzman *et al* (43) undertook an evaluation of the physiological distribution of both cyclizine and chlorcyclizine. The tissue distribution of chlorcyclizine (50mg/kg intraperitoneally) and cyclizine (25mg/kg intraperitoneally) administered to male rats three to four hours prior to sacrificing was studied. It was noted that both drugs have a similar distribution and are markedly localized in the tissues, especially the lung, spleen, liver and kidney with levels 20 to 110 fold that found in the plasma.

Analysis of tissue extracts from the liver, kidney, lung, spleen and brain tissue of female rats administered chlorcyclizine (50mg/kg orally) for 3, 7 and 14 days showed a continuous increase in the concentration of chlorcyclizine and its metabolites (63). The tissue distribution observed here confirms that observed by Kuntzman an co-workers (43).

Studies to observe the distribution of chlorcyclizine and possible teratogenic effects involved the administration of chlorcyclizine-^{14}C to pregnant mice (64). Radioactivity was observed in the liver, kidney, lung, the 12.5 day embryo and in 17.5 day foetal tissue. The high concentration of

chlorcyclizine in the lung may be due to accumulation in the acidic environment associated with lung tissues. The pK_a of chlorcyclizine is approximately 7.9 and thus it is expected to accumulate in an acidic environment. The low concentration in the embryo at all time intervals may be a result of active secretion of the drug by the yolk sac into the uterine lumen or, due to foetal tissues being more alkaline than that of the mother. The drug being a weak base would tend not to accumulate in an alkaline environment.

A comparative assessment of the distribution of tritiated hydroxyzine and hydroxyzine methiodide-^{14}C in rats was performed (65). Rats were administered the respective compounds by intraperitoneal administration and the distribution of hydroxyzine into the tissues was found to be rapid. The highest specific radioactivity was observed in the lungs, followed by fat, liver, spleen and kidneys. The distribution of the methiodide was similar to that of hydroxyzine but was restricted to fewer organs with lower specific radioactivity.

Pharmacokinetic studies on hydroxyzine in both dogs (66) and humans of different ages and disease states (53,54,55,56) indicate high apparent volume of distribution values (V_d). The V_d determined in these studies are listed in Table 1.4.

Table 1.4 Apparent Volume of Distribution of Hydroxyzine in Dogs and Humans.

Subject	Apparent Volume of Distribution (ℓ/kg)	Reference
Beagle Dogs	4.6 ± 0.8	Simons *et al* (66)
Young healthy adults (n=7)	16.0 ± 3.0	Simons *et al* (53)
Children with dermatitis (n=11)	18.5 ± 8.6	Simons *et al* (54)
Elderly adults (n=9)	22.5 ± 6.3	Simons *et al* (55)
Adults with cirrhosis (n=8)	22.7 ± 13.3	Simons *et al* (56)

V_d is a mean value of those determined in a study over 150 days with six dogs.

Meclizine hydrochloride appears to follow two compartment pharmacokinetics when administered as an intravenous bolus dose (1mg/kg) to a dog (67). It appears as though there is a rapid distribution phase and the apparent volume of distribution of the ß phase was 64ℓ.

Generally for a 70kg adult an apparent volume of distribution of 0.58ℓ/kg is an indication that a drug is distributed in the whole body fluid (68). Therefore any apparent volume of distribution in excess of 0.58ℓ/kg is an indication that a drug is extensively distributed to tissues or bound to plasma or tissue proteins. Physiological evidence published by several researchers indicate that the piperazines can be expected to exhibit large apparent volumes of distribution.

1.3.3 **Metabolism**

In general, the primary site for the metabolism of antihistamines is the liver (15). Metabolism of drugs may be divided into non-synthetic (Phase 1) or synthetic (Phase 2) reactions (68). The result of either Phase 1 or Phase 2 reactions is the formation of compounds usually less pharmacologically active than the parent compound. Oxidation, reduction or hydrolysis comprise Phase 1 reactions and conjugation Phase 2 reactions. The most common of conjugation reactions occurs with acetate, sulphate, glycine or glucuronic acid (69).

Kuntzman *et al* (43) have determined that both cyclizine and chlorcyclizine are metabolised to inactive demethylated derivatives, norcyclizine and norchlorcyclizine. *In vitro* and *in vivo* studies in rats showed sex differences in the metabolism of chlorcyclizine. Male rats appeared to demethylate chlorcyclizine faster than the female rat. Administration of cyclizine and chlorcyclizine to female rats revealed that the demethylated metabolites disappear at different rates, with norcyclizine disappearing faster than norchlorcyclizine. The implication is that chronic dosing of cyclizine would not lead to norcyclizine accumulation in the tissues as norchlorcyclizine would after chlorcyclizine administration. This was confirmed by the presence of high levels of norchlorcyclizine in all tissues studied.

Both cyclizine and chlorcyclizine may induce liver enzyme microsomal activity in experimental animals (9) and chlorcyclizine has been shown to stimulate its own metabolism in dogs (70). To date stimulation of self metabolism for cyclizine has not been proved, but should not be discounted.

N-oxide formation was postulated as a new route for inactivation of chlorcyclizine (71) after evidence of the N-oxide of the drug was found in the urine of rats and humans. The N-oxide is inactive when tested for antihistaminic activity *in vitro*. Kuntzman *et al* (71) suggest that although the N-oxide is a metabolite of chlorcyclizine it is not an intermediate in the dealkylation reaction to norchlorcyclizine. This was evident after no norchlorcyclizine was detected when chlorcyclizine N-oxide was incubated with a rat liver microsomal enzyme system which extensively demethylates chlorcyclizine. However, the administration of chlorcyclizine N-oxide *in vivo* resulted in the

formation of norchlorcyclizine which may be explained by the initial reduction of the N-oxide to chlorcyclizine followed by demethylation.

Extracts of liver, kidney, lung, spleen and brain tissue of female rats sacrificed 24 hours after receiving chlorcyclizine (50mg/kg orally) for 3, 7 or 14 days showed a continuous increase in the concentrations of chlorcyclizine, norchlorcyclizine and of N-(*p*-chlorobenzhydryl)-ethylenediamine (63). The latter compound was formed by partial degradation or cleavage of the piperazine ring which is preceded by dealkylation. Recently, homchlorcyclizine was shown to exhibit enantioselective pharmacokinetics with respect to its metabolism (72).

N^+-Glucuronidation has been investigated as a possible route for the metabolism of the H_1-antihistamines in humans (73). All classes of antihistamines were studied and in eight of the nine drugs studied, metabolism via the N^+-glucuronidation pathway occurred. Cyclizine N^+-glucuronide recovered in the urine accounted for 14.3 percent of the dose (50mg cyclizine lactate solution orally) administered. A recent study (74) revealed that cyclizine N^+-glucuronide was excreted in both the urine and faeces of volunteers after the same dose. The proportion of the dose excreted as the glucuronide in urine and faeces was 15.3 ± 3.3 percent ($n=10$) and 2.6 ± 3.6 percent ($n=4$) respectively. The corresponding amounts of cyclizine recovered unchanged in the urine and faeces were 1.4 ± 0.7 percent ($n=10$) and 2.2 ± 1.2 percent ($n=10$) respectively. The volunteers in the study were five healthy caucasian and five oriental males. The results appear to show inter-ethnic differences in the excretion of the glucuronide in the faeces as only four of the oriental males had cyclizine N^+-glucuronide in their faeces.

Pong and Huang (65) isolated five metabolites of hydroxyzine, mainly as glucuronides of the diphenylmethane derivatives. In addition, hydroxyzine, may be metabolised by a cytochrome P_{450} system, in the liver, to an active carboxylic acid metabolite, cetirizine, and other metabolites (75).

As with other compounds of this group meclizine also undergoes extensive biotransformation prior to excretion. Following administration to rats one of the products of the metabolic process was identified as norchlorcyclizine (76). Five other nonbasic metabolites were also isolated, but only the basic metabolite, norchlorcyclizine, appears to cross the placental barrier implicating the compound as a teratogen. The low recovery of norchlorcyclizine in tissues suggest that the drug may be further metabolised. In this regard, Conney and Klutch (77) have shown that an enzyme present in liver microsomes is capable of removal of N-alkyl groups via an oxidative process. Benzyl groups have not been implicated in this reaction, however, the presence of high levels of norchlorcyclizine in meclizine treated rats suggest the involvement of this enzyme system or a similar one in meclizine

metabolism. As with chlorcyclizine metabolism, Gaertner *et al* (63) also noted the presence of the metabolite N-(*p*-chlorobenzhydryl)-ethylenediamine, produced by cleavage of the piperazine ring.

1.3.4 Elimination

Elimination can be described as a combination of both excretion and metabolism. The main route of excretion is via the kidneys and the mechanism by which most drugs are eliminated from the body is biotransformation (78). Two concepts of importance in drug elimination are elimination half-life and clearance. The elimination half-life of a drug is defined as the time in hours necessary to reduce parent drug concentration in the blood, plasma or serum by one half after equilibrium is reached. Clearance may be defined as an hypothetical volume of distribution in millilitres of unmetabolized drug which is cleared per unit of time (ml/min or ml/hour) by any pathway of drug removal (68).

Administration of both cyclizine and chlorcyclizine to dogs revealed that cyclizine disappeared faster from plasma than chlorcyclizine (43). Plasma protein binding studies showed that the chlorinated derivative and its metabolite were more extensively bound to proteins than cyclizine and its metabolite. Extrapolation to tissue proteins may offer an explanation for the rapid disappearance of cyclizine and norcyclizine from the tissues, as more of the compound would be available for metabolism by liver enzymes due to lower binding. Excretion of both chlorcyclizine and its metabolite in the urine of rats after oral administration is low, with only 5 percent of the dose recovered as either compound.

The N-oxide metabolite of chlorcyclizine has been isolated in the urine (4.2% of the dose) of female rats administered chlorcyclizine (50mg/kg orally) and some evidence exists of its excretion in humans (71).

Cyclizine and its metabolite, norcyclizine, have been found to disappear more rapidly from the rat,dog and man than the structurally similar compound chlorcyclizine and its metabolite, norchlorcyclizine (79). Forty eight hours post administration of chlorcyclizine and cyclizine to rats, high levels of the norchlorcyclizine were still present in lung tissue, whilst only small amounts of norcyclizine were present at the same time. Similarly in dogs, the plasma half-lives of cyclizine and norcyclizine were much shorter than chlorcyclizine and norchlorcyclizine. The retention of norcyclizine and norchlorcyclizine after administration of the parent compounds (50mg three times a day for 6 days) was similar and the plasma half-life of norchlorcyclizine was 6 days and that of norcyclizine less than 1 day. Similar results were observed when urine samples were studied. More extensive binding of norchlorcyclizine over norcyclizine to plasma and tissue proteins has been cited

as a possible explanation for the preferential retention of the compound. This has been confirmed by the fact that a more concentrated lung homogenate (10%) is necessary to bind a similar percentage of norcyclizine (54%) and extrapolation to determine percentage bound to 100 percent lung tissue indicates the free concentration of norcyclizine to be four fold that of norchlorcyclizine. The extent of binding may offer an explanation for the longer retention, in the body, of the chlorinated derivative.

The persistence of chlorcyclizine, norchlorcyclizine and N-(*p*-chlorobenzhydryl)-ethylenediamine was noted by Gaertner *et al* (63). They reported that after administration of chlorcyclizine to female rats for 14 days, there was a rapid decline in parent drug concentration and that four days after termination of treatment the compound was inconsistently present. The reported half life of norchlorcyclizine was approximately three days and the ethylenediamine derivative was present for at least 14 days.

The elimination of cyclizine following an intravenous dose (50mg cyclizine hydrochloride) to a human volunteer was monitored (60). A delay in the excretion was noted at 30 minutes after the dose, a phenomenon that is consistent for basic drugs.

Kuntzman *et al* (80) found norcyclizine levels between 4ng/ml and 22ng/ml in four human subjects that received 50mg cyclizine three times a day for six days. The 24-hour urine collected on the day following the completion of dosing contained on average 1.1mg norcyclizine, or approximately 0.8 percent of the daily 150mg dose. After oral administration of cyclizine (50mg cyclizine hydrochloride) the blood levels declined in a biphasic manner, with estimated elimination half-lives of 7 hours and 24 hours for the early and late phases respectively (46). In the same, study twenty four hour urinary excretion data revealed no norcyclizine to be present and a peak urine concentration for cyclizine 4 hours after the dose of 12.5ng/ml. During the entire 24-hour urine collection period only 0.01 percent of the administered dose was excreted as unchanged cyclizine. Calculated elimination half-lives in a pilot study which also showed biphasic elimination for cyclizine after an oral dose (50mg cyclizine hydrochloride) were consistent with the above. The estimated half-lives were 6 hours and 22 hours for the early and late phases respectively (47). Urine collected for 12 hours after the dose contained both unchanged cyclizine and norcyclizine in concentrations 10 fold that previously reported by Griffin and Baselt (46).

Elimination half-lives for cinnarizine from three different studies are listed in Table 1.3. The removal of cinnarizine from the body is rapid with an average elimination half-life of 3.47 hours for all studies. One would expect the effects of cinnarizine to diminish rapidly after administration.

Fouda *et al* (52) calculated the elimination half-life of hydroxyzine from rate constants obtained by computer modelling of data to an open one compartment model. The mean half-life of 3 hours, in the four subjects, was similar to that of a chemically related drug, meclizine (57).

Changes in serum half-lives and clearance rates after chronic administration (150 days) of hydroxyzine (0.7mg/kg intramuscularly) to dogs were studied to determine whether subsensitivity of the H_1-receptor antagonists can be attributed to autoinduction of enzyme systems or increased clearance rates (66). Mean serum half-lives on day 30, 60 and 120 were significantly ($p < 0.05$) longer than that of 2.4 hours obtained on day 1. The mean clearance values obtained on days 30, 60, 90, 120 and 150 were significantly ($p < 0.05$) slower than the value of 25.12 ml/min/kg obtained on day 1, but were not significantly different from each other. These results add to the evidence that subsensitivity of the H_1-receptor antagonists are not due to autoinduction of enzyme systems or to more rapid clearance of the drug and lower concentrations of the drug in serum and tissue.

Gengo *et al* (75) performed pharmacokinetic studies on 12 healthy volunteers. The elimination followed a bi-exponential decline similar to that of cyclizine and determined the mean elimination half-life to be 14 hours. The clearance estimate for hydroxyzine in these volunteers was $69 \pm 25 \ell$/hr. Other half-life and clearance values obtained from pharmacokinetic studies on hydroxyzine in both dogs (66) and humans of different ages and disease states (53,54,55,56) are listed in Table 1.5.

Table 1.5 Clearance and Serum Half-Life Values for Hydroxyzine.

Subject	Clearance (ml/min/kg)	$t_{½}$ (hr)	Reference
Beagle Dogs	*17.0 ± 3.8	*3.2 ± 0.7	Simons *et al* (66)
Young healthy adults (n=7)	9.8 ± 3.2	20.0 ± 4.1	Simons *et al* (53)
Children with dermatitis (n=11)	32.1 ± 11.1	7.1 ± 2.3	Simons *et al* (54)
Elderly adults (n=9)	9.6 ± 3.2	29.3 ± 10.1	Simons *et al* (55)
Adults with cirrhosis (n=8)	22.7 ± 13.3	36.6 ± 13.1	Simons *et al* (56)

* Mean values determined in a study over 150 days with six dogs.

The half-life of hydroxyzine appears to be longer than that of cinnarizine and meclizine. Hydroxyzine, is eliminated more rapidly by children than by adults which is also true for another H_1-receptor antagonist, chlorpheniramine (81). The lipophilic nature of hydroxyzine and the increase in adipose tissue, due to normal ageing processes, may account for the increase in half-life

in the elderly subjects (55) due to more extensive distribution of the drug. The longer half-life in adults with cirrhosis is likely to be a combination of impaired hepatic function and ageing (56).

The terminal elimination pharmacokinetics of meclizine following administration via oral, intranasal or intravenous routes were found to be similar within each species studied (59). Mean half-life values obtained were 62.3 ± 6.18 minutes and 5.38 ± 0.25 hours for the rat and dog respectively. The terminal half-life (3 hours) of meclizine in a dog administered the drug (1mg/kg) was slightly shorter than the five hours reported (67).

Jun *et al* (67) also monitored the urinary excretion profile of meclizine in a 70kg male and showed that approximately 3.2% of the dose was excreted unchanged in a 24 hour period, indicating that extensive biotransformation of meclizine occurs.

1.3.5 Cyclizine: Pharmacokinetic Summary

Cyclizine hydrochloride appears to be rapidly absorbed with t_{max} occurring at between two and three hours and peak concentrations of approximately 50ng/ml to 70ng/ml (46,47). Despite the administration of an intravenous dose of cyclizine hydrochloride (60) the absolute bioavailability of cyclizine was not determined.

Cyclizine was found to be extensively distributed when administered intraperitoneally (25mg/kg) to male rats with levels 20 to 110 fold that found in plasma, in the lung, spleen, liver and kidney (43). The extensive physiological distribution suggests that the apparent volume of distribution of cyclizine would be high (in the region of 2ℓ/kg - 5ℓ/kg). The pK_a of cyclizine hydrochloride is approximately 8.2 (5). Evidence that chlorcyclizine ($pK_a \approx 7.9$) was found in high concentrations in lungs (64) suggest that cyclizine would also distribute to the acidic environment of the lung.

Metabolic inactivation of cyclizine takes place in the liver, primarily by demethylation, to an inactive metabolite norcyclizine (43). As with any drug that is metabolised by the liver, cyclizine may induce liver enzyme activity and the possibility of stimulation of self metabolism should not be rejected (9). Chlorcyclizine, a chemically related compound, has been shown to exhibit differences in metabolism between males and females (43), and therefore these differences should not be overlooked when cyclizine is to be used clinically. High levels of norcyclizine were present shortly after chronic administration of cyclizine (75mg/kg orally) to female rats for 6 weeks but the metabolite did not accumulate to any extent, indicating rapid elimination of norcyclizine (43). A similar phenomenon would be expected on chronic adminstration of cyclizine to humans.

Two other routes of metabolic inactivation of chlorcyclizine have been published. These postulate the formation of the N-oxide (71) and a product (N-(*p*-chlorobenzhydryl)-ethylenediamine) formed by cleavage of the piperazine ring (63). These two routes of inactivation may also account for the disappearance of cyclizine from the body. N^+-glucuronidation is a further route of inactivation of cyclizine accounting for 14.3 percent of an administered dose (50mg cyclizine lactate solution orally) to a human volunteer (73). More recently, cyclizine N^+-glucuronide has been isolated in the urine and faeces of ten human volunteers that received 50mg of cyclizine lactate solution. The proportion of the dose recovered as N^+-glucuronide was 15.3 percent and 2.6 percent in the urine and faeces respectively (74). The excretion of parent drug in urine and faeces in the same volunteers accounts for 1.4 percent and 2.2 percent of the administered dose.

It is apparent that urinary excretion of cyclizine as either parent drug or demethylated metabolite, norcyclizine, is low (ie. $<$ 2 percent of the administered dose) (46,47,74,80). A larger percentage of the dose is excreted as cyclizine N^+-glucuronide (73,74).

Cyclizine elimination from blood appears to follow a biphasic pattern with estimated half-lives for the early and late phases in two independent studies being 7 hours and 24 hours (study 1) (46) and 3 hours and 10 hours (study 2) (47) respectively.

CHAPTER 2

ANALYSIS OF CYCLIZINE

The quantitative analysis of cyclizine (a weak base) in dosage forms has been accomplished using a variety of analytical techniques. These include the use of fluorometry (82), gas chromatography (83), colorimetry (84), titrimetry (85), second-derivative ultraviolet spectrophotometry (86) and reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection (87,88). Furthermore, several methods have been published for the assay of cyclizine in both serum and urine using colorimetric methods (43), derivatization with tritiated acetic anhydride (79) and gas-liquid chromatography (46,60) and HPLC (89). These methods of analysis for cyclizine have limitations with regards complexity, sensitivity and ruggedness.

In vitro testing ensures that quality products are released for use to the public. These tests not only ensure that the correct quantity of drug has been incorporated into a dosage form, but also, from dissolution study data, conservative predictions relating to the performance of the dosage form when used therapeutically may be inferred. Analysis of biological samples (such as serum and urine) however, are essential for pharmacokinetic characterization of drugs. In order to achieve the necessary sensitivity and selectivity to quantitate the low levels of drugs found after therapeutic doses, a suitable analytical technique is required.

Chromatography, which may be defined as the separation of solutes by passing a sample through a stationary phase by a flowing (or mobile) phase is such a technique (90). Of all the chromatographic techniques, reversed-phase HPLC has the required sensitivity, selectivity and versatility to provide fast and efficient drug analysis for dosage form and biological sample assessment. In addition, HPLC has a further advantage in that it can be used for the determination of polar and thermolabile compounds (91). An HPLC method was therefore developed for dosage form and biological sample analysis.

2.1 ANALYTICAL METHOD DEVELOPMENT

2.1.1 Principles of Reversed-Phase Liquid Chromatography

HPLC for compounds of low molecular weight may be classified as normal phase or adsorption chromatography, reversed-phase chromatography or ion-exchange chromatography (92) and the requirements for each technique are specific. For example, in reversed-phase chromatography, the stationary phase is non-polar and the eluent polar. The majority of compounds of pharmaceutical interest are relatively non-polar and therefore most HPLC analyses in pharmaceutical research are predominantly reversed-phase in nature (93). Reversed-phase chromatography is usually performed on a hydrophobic bonded stationary phase with polar solvents such as water and mixtures of water and water-miscible organic solvents such as acetonitrile or methanol (94).

Reversed-phase chromatographic separation procedures involve several mechanisms, the most conventional of which relies on the hydrophobic characteristics of the solute or analyte of interest and the properties of the stationary phase. Other techniques make use of an ionic substance to control pH (ion-suppression) or in the case of strong acids or bases the use of a counterion that renders the compound of interest electrically neutral (ion-pair chromatography) enabling separation by reversed -phase HPLC. The addition of metal ions or chelates to the mobile phase has been used with some success with special reference to method selectivity (94).

The bonded phases are classified according to the length of the organic side chain which may range from two carbons to eighteen. Commonly used stationary phases are of 5μm or 10μm in diameter although smaller diameters are available for use (94). Stationary phases for reversed-phase chromatography are prepared by the reaction of *n*-alkylchlorosilanes with silanol functional groups of a silica support. The resultant effect is the formation of a relatively hydrophobic surface on the hydrophillic surface of the silica. The degree to which the reaction is completed varies the hydrophobic/hydrophillic characteristics of the stationary phase and the subsequent retention and separation of many compounds by silanophilic or solvophobic retention mechanisms (95). The exact method by which this occurs is not clear. The solvophobic or hydrophobic mechanism of retention involves partitioning of compounds from a relatively hydrophillic environment (the mobile phase) into a relatively hydrophobic environment (the stationary phase) and silanophilic retention mechanisms involve hydrogen bonding and/or ionic interactions between the compound and the residual ionised silanol groups on the surface of the stationary phase (92). The mechanism of retention of a compound is peculiar to that specific substance and despite the high degree of lipophilicity of a C_{18} stationary phase, may occur by solvophobic or silanophilic mechanisms (96).

In addition, particle size of packing materials and column packing technique have been implicated in determining the separating power of a particular column (97). It follows therefore that the elution of any compound from reversed-phase packing materials is dependent upon it's physico-chemical properties (for example pK_a and partition coefficient), the column (material and particle size) and composition of the mobile phase especially if an organic-aqueous mixture with either amine modifier or buffer is used. The chromatographic behaviour of compounds has been found to be predictable on the basis of pK_a and partition coefficient for acidic and neutral drugs, however reversed-phase column efficiency for basic drugs such as cyclizine is poor resulting in peak tailing and poor peak shape (98). Separations may be affected and improved by modification of the composition of the aqueous phase and adjustment of the organic-aqueous ratio (94). It is therefore feasible that even for basic compounds reversed-phase column efficiency can be improved by careful consideration of all aspects of the chromatographic system.

2.1.2 Selection of Analytical Column

Cyclizine is a weakly basic piperazine compound with a pK_a of 8.2. The two benzene rings ensure some degree of lipophilicity to the molecule and the nitrogen presence in the piperazine ring allows for some degree of polarity. These characteristics ensure some degree of retention of the drug on a reversed-phase packing material. Few HPLC methods have been published for the analysis of cyclizine. On the basis of previous work done in our laboratory (89) , initial studies were performed using a 10μm C_{18} packed column for both *in vitro* and *in vivo* testing.

The stainless steel analytical columns (25cm x 3.9mm i.d.) were packed in the laboratory with Techsil® reversed-phase micro-particulate bonded (10μm) octadecylsilane (C_{18}) material utilizing a slurry technique similar to that described by Melander and Horváth (99) and a Shandon column packing apparatus (Shandon Southern Products Ltd., Cheshire, United Kingdom). Each column packed in our laboratory was subjected to an efficiency test by injection of a test mixture containing benzamide, benzene, benzophenone and biphenyl onto the column. The separation was achieved with a mobile phase of acetonitrile-water (75:25) at a flow rate of 1.0ml.min^{-1}. The compounds were detected at 254nm. A typical chromatogram of the separation achieved is depicted in Figure 2.1.

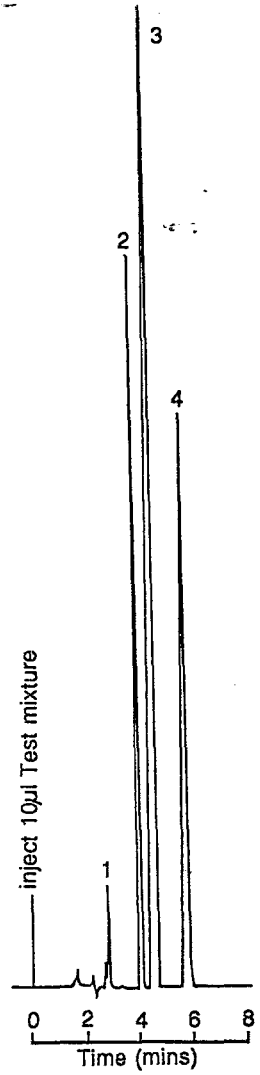


Figure 2.1 Typical chromatogram of a test mixture containing benzamide (1), benzene (2), benzophenone (3) and biphenyl (4) after chromatography on a 10μm C_{18} analytical column packed in our laboratory.

Chromatographic system efficiency is usually expressed quantitatively as the number of theoretical plates and can be calculated using either equation 2.1 or 2.2 (100):-

$$N = 5.54 \left(\frac{t_R}{W_{1/2}} \right)^2 \qquad \text{Eq. 2.1}$$

$$N = 16 \left(\frac{t_R}{W} \right)^2 \qquad \text{Eq. 2.2}$$

where N = the number of theoretical plates,
t_R = the retention time of a test peak,
$W_{½}$ = the peak width at half peak height, and
W = the peak width at the baseline.

The calculation of theoretical plate number for analytical columns using the above formulae is based on the assumption that the peak of interest is Guassian, which is not often true for most drugs. The calculation does however give an indication of the possible efficiency and performance of a column based on the theoretical plate number. Columns packed in our laboratory and exhibiting plate counts of 5000 or greater were used.

2.1.3 HPLC Apparatus

The modular chromatographic system used consisted of a Waters Model M 6000A dual piston constant flow pump (Waters Assoc., Milford, MA, U.S.A.), an automated Waters Intelligent Sample Processor Model 710B (WISP, Waters Assoc., Milford, MA, U.S.A.), a variable wavelength UV absorbance detector Model SP 8480 XR (Spectra-Physics Inc., San Jose, CA, U.S.A.) and a dual-pen Model 561 strip chart recorder (Hitachi Ltd., Tokyo, Japan). The mobile-phase was constantly degassed using an in-line degasser Model ERC-3510 (Erma Optical Works Ltd., Tokyo, Japan). The analytical column was a 25cm x 4.1mm i.d. stainless-steel column, packed with Techsil 10μm octadecylsilane (C_{18}) material (HPLC Technology, Wilmslow, United Kingdom). The column was maintained at 30°C with a Model LC-22 temperature controller (Bioanalytical Systems INC., West Lafayette, IN, U.S.A.).

2.1.4 Chemicals

All chemicals used were at least of analytical reagent grade. Acetonitrile (UV cut off 190nm) was distilled-in-glass UV grade (Burdick and Jackson, Muskegon, MI, U.S.A.). AnalaR® phosphoric acid (85%) and sodium hydroxide pellets were obtained from BDH Chemicals Ltd. (Poole, England). Cyclizine base and cyclizine hydrochloride were obtained from Wellcome (Pty) Limited (Johannesburg, South Africa) and norcyclizine (an inactive demethylated metabolite of cyclizine) from the Wellcome Foundation Limited (Dartford, United Kingdom). Protryptiline hydrochloride was obtained from MSD Laboratories (Johannesburg, South Africa). Water used for buffer preparation, extraction and chromatography was initially purified by a reverse-osmosis Milli-RO 15 Water Purification System (Millipore, Bedford, MA, U.S.A.) that consisted of a Super-C carbon cartridge, two Ion-X ion exchange cartridges and an Organex-Q cartridge. The water was filtered through a 0.22μm Millipak stack filter, prior to use.

2.1.5 Internal Standard

The use of an internal standard is one method used to improve the accuracy of an analytical method. The choice of the standard is therefore critical to ensure maximum optimization of the method. The internal standard method compensates for varying injection volumes and day to day instrumental changes thereby promoting method accuracy (101). Salient features of an internal standard are that the compound should be similar in structure to unknown compounds of interest and behave in a similar manner chromatographically. In addition the compound should exhibit the same response as the unknown compounds with respect to detection systems used. Of the chromatographic methods published for analysis of cyclizine, chlorcyclizine (60), diphenhydramine (46), protryptiline (89) and ethyl *p*-hydroxybenzoate (88) have been used as internal standards. Protryptiline hydrochloride was selected as the internal standard , for this assay, based on previous work done in our laboratory (89).

2.1.6 Mobile-Phase Selection

An evaluation of the UV absorption spectrum of cyclizine (Figure 1.4) revealed that the wavelength of maximum absorption is in the vicinity of 200nm. There is a smaller maximum at 230nm. This is an indication that in order to use UV spectrophotometry for the analysis of cyclizine, low wavelengths in the ultra-violet range are necessary. This necessitates the use of solvents and buffers in the mobile-phase that have an ultra-violet cut-off below 200nm. Acetonitrile (UV cut-off of 190nm), water, phosphoric acid and sodium hydroxide were used in the preparation of a suitable mobile-phase (89). Resolution and acceptable retention times were obtained using a mobile-phase of acetonitrile-phosphate buffer 0.05M pH=3 (2:3). The mobile-phase buffer was prepared by the addition of 5.28ml orthophosphoric acid to 2ℓ of HPLC grade water and adjusting the pH to 3.00 with sodium hydroxide pellets. The use of salts in the preparation of the buffer was avoided due to possible absorbance of some ionic species at low UV wavelengths. Following mixing of the acetonitrile and buffer, the mobile-phase was degassed under vacuum and filtered through a 0.45μm HVLP Millipore filter prior to use. On-line degassing maintains UV detector sensitivity by reducing baseline drifts and random noise that may be generated by gases dissolved in the mobile phase.

2.1.7 UV Detection of Cyclizine, Norcyclizine and Internal Standard

The λ-max for cyclizine (Figure 1.4), is 200nm. It is well known that at low wavelengths many compounds exhibit UV absorption thereby decreasing selectivity of analytical methods. Due to the low molar absorbtivities of cyclizine and norcyclizine at wavelengths normally used for drug analysis (220nm and 254nm) it was necessary to monitor the eluate at 200nm. The detection of the internal

standard does not have to occur at λ-max for the compound as sufficient material may be used to obtain an adequate response at the desired attenuation.

2.1.8 Chromatographic Conditions

1) Mobile-phase flow rate	- 1.0ml.min^{-1}
2) Column	- Techsil C_{18} (10μm)
	- Length 250mm
	- I.D. 4.1mm
3) Column temperature	- 30°C
4) Column pressure	- 7 x 10^{6}Nm^{-2}
5) Retention times	- Norcyclizine *ca.* 4min
	- Cyclizine *ca.* 5min
	- Internal standard *ca.* 7.5min
6) Detector settings	- λ=200nm
	- Absorbance units full scale setting 0.01
7) Injection volume	- 5-10$\mu\ell$
8) Chart speed	- 5mm/min
9) Chart recorder input	- 10mV full scale

A typical chromatogram of an aqueous sample of norcyclizine, cyclizine and protryptiline is depicted in Figure 2.2.

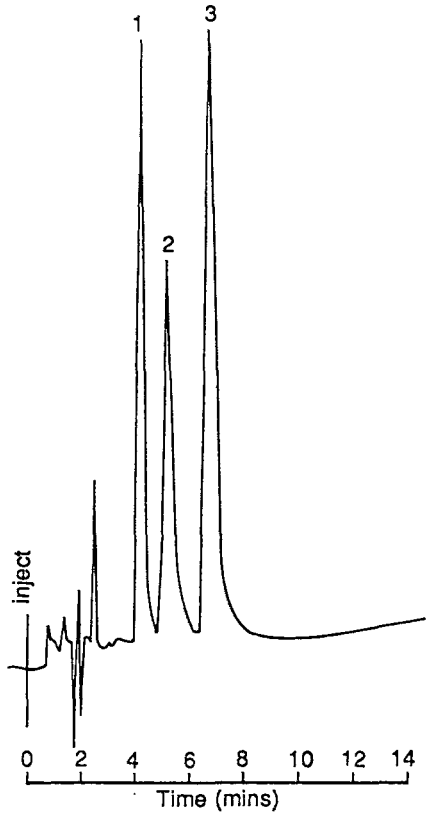


Figure 2.2 Typical chromatogram obtained at 200nm on a 10μm C_{18} analytical column of an aqueous sample of norcyclizine (1); on column load = 23ng, cyclizine (2); on column load = 6.5ng, and protryptiline (3); on column load = 25ng. Other chromatographic conditions are as specified in § 2.1.8 and mobile-phase as specified in § 2.1.6.

2.2 EXTRACTION OF NORCYCLIZINE AND CYCLIZINE FROM SERUM AND URINE

The analysis of drugs from biological fluids requires a sensitive, selective analytical technique to ensure an accurate representation of what is occurring in the body. Previously published methods for the analysis of cyclizine in serum and cyclizine and norcyclizine in urine lacked both the sensitivity and selectivity desired for the accurate assessment of the disposition and pharmacokinetics of the drug. Prior to the determination of a drug in a biological matrix a suitable extraction technique is necessary to remove the compound of interest from contaminating proteins and salts that may be present, followed by a concentration step to ensure detection of low or trace levels of the compound.

Previously most sample preparations involved solvent extraction of the biological matrix. Essentially this necessitated the partitioning of drug from a sample into an added immiscible solvent (102). This technique is dependent on changes in pH of the aqueous phase and the choice of immiscible solvents and extractions may become quite complex procedures involving back extractions. In addition, operator technique, emulsion formation and solvent wastage pose problems. Several of the published methods for the determination of cyclizine in biological matrices involved liquid-liquid back extraction procedures (46,60,89).

A useful and effective alternative to liquid-liquid extraction is the recovery of drugs by adsorption methods (103) and with the development of bonded silica chemistry, sorbent extraction has become a powerful tool for drug isolation and purification (104). Sorbent extraction for sample preparation has developed rapidly during the 1980's and on-line sorbent extraction has become more popular with the development of automated systems (105). Several advantages exhibited by this technique over liquid-liquid extraction include increased speed of sample throughput, improved selectivity of the extraction and the ability to use a wide variety of extraction conditions to achieve the desired separation. Solid-phase extraction (SPE) methods are approximately 12-fold less time consuming and five-fold less costly than liquid-liquid extractions (106). Liquid-solid extraction separates different solutes by utilizing the principles of modern liquid chromatography. Essentially, the fluid sample passes over an adsorbent stationary phase and the analytes are separated according to the degree of partitioning or affinity it has for the stationary phase. In this manner compounds of interest may be separated from undesirable contaminants in the sample. Stationary phases for SPE vary according to the nature of the functional group attached to the silica support and because of this can be used for the isolation of many different compounds. A solid-phase cation exchange procedure for the determination of cyclizine as one of 14 drugs from greyhound urine has been reported (107).

On the basis of previous work done in our laboratory with the analysis of weakly basic macrolide antibiotics such as erythromycin, oleandomycin and josamycin (108,109,110) using solid-phase extraction (SPE) methods a SPE method was developed.

2.2.1 Sample Matrix Considerations

The nature of each matrix that is sampled will pose specific problems for the analyst with respect to clean up, recovery and reproducibility of the assay. For pharmacokinetic studies biological fluids analyzed are serum/plasma and urine and consideration of their composition is essential to provide a reproducible analytical method.

2.2.1.1 Serum

The isolation of any compound from serum involves consideration of the presence of proteinaceous materials for one of two reasons:-

1. The affinity of the compound of interest for protein, and
2. The retention of protein in the sample and subsequent difficulty in analysis.

Sample pre-treatment prior to extraction is therefore essential in order to minimize chromatographic interference from endogenous compounds. Attempts to obtain protein free samples by use of dialysis and ultrafiltration may also cause loss of the compound of interest (111) and the measurement of free drug will underestimate the total drug present. The precipitation of proteins in samples for SPE methods is necessary to prevent clogging of the columns. The use of materials such as perchloric and trichloroacetic acids have been used to precipitate proteins (112). Precipitation of the protein causes destruction of the drug-protein complex releasing the drug and allowing the total drug concentration to be determined. A commonly used method to prepare protein free serum samples for extraction is to facilitate precipitation of proteins by mixing equal volumes of serum and water miscible organic solvents such as acetonitrile or doubling the relative volume of acetonitrile in cases of incomplete precipitation followed by centrifugation (113,114). This technique has been used successfully in our laboratories for the precipitation of serum proteins prior to the analysis of the macrolide antibiotics erythromycin, oleandomycin and josamycin (108,109,110). One consolation for the analyst is that despite the fact that serum and plasma are complex matrices, their composition is relatively stable with factors such as pH, total protein and salt concentrations remaining constant from subject to subject (112). An important consideration however is that the lipid content may vary considerably depending on time and nature of meals given throughout the duration of a clinical study.

2.2.1.2 Urine

Analytical problems encountered with urine samples are unlikely to be due to protein and lipid content but rather due to variable composition, volume and pH of the samples (112). The pH range of normal urine is 5.5 to 7.0. The pH of the urine could and does influence the nature of the excreted drug. For example, a weakly basic drug such as cyclizine will be more efficiently excreted in acidic urine. In addition, variable volumes excreted during the collection intervals may pose problems with respect to limits of sensitivity especially if large volumes are excreted. The presence of large quantities of water soluble solutes in urine whilst being no problem for liquid-liquid extraction may alter the composition of wash solvents in SPE thereby promoting unnecessary loss of drug particularly if elution from the sorbent is buffer sensitive (115).

2.2.2 Method Development

There are a wide variety of stationary phases available for use by the analyst in SPE procedures. Bonded silica extraction columns are becoming more popular and may be divided into three groups, namely non-polar, polar and ion-exchange. Perusal of the literature revealed that most SPE procedures use non-polar sorbents of which C_{18} is the most popular, due to the fact that most compounds exhibit some degree of lipophilicity ensuring partial if not total retention of the compound of interest on the bonded silica matrix.

The use of SPE for the extraction of basic compounds and particularly retention mechanisms and factors affecting retention have received much attention over the last few years. The use of C_{18} sorbents suggests that conventional reversed-phase retention mechanisms would predominate. Ruane and Wilson (116) have shown that attempts to elute basic compounds retained on C_{18} with a strong eluotropic solvent such as acetonitrile were unsuccessful. The use of buffered solvent systems successfully eluted the compounds of interest suggesting that retention is a combination of conventional reversed-phase mechanisms and ionic interactions between the positively charged drug molecules and the negatively charged silanol groups remaining on the surface of the silica gel after silylation. These results have recently been confirmed by Law *et al* (117) who have demonstrated that cation exchange plays a significant role in the retention of basic compounds on reversed-phase extraction cartridges and that in order to elute compounds of interest both reversed-phase and ionic interactions need to be overcome. This is usually achieved by use of an aqueous/organic solvent with a suitable competing cation. The role of hydrogen bonding in SPE of polar basic drugs has been elucidated by inclusion of strong hydrogen bond acceptors in the eluent or increasing the water component of the eluent (118).

An important consideration in the extraction of any compound is the possible effects the sample matrix may have on the procedure. Law and Weir (119) have indicated that the success of SPE for basic compounds is dependent to a large degree on the protein binding characteristics of the compound in question as well as the cationic components of the serum/plasma.

C_{18} sorbents used in extraction columns are commonly used for the isolation of drugs from biological matrices as they have the ability to retain non-polar compounds of varied structure and allow the polar materials to wash off the column. Due to low selectivity final extracts may not be as pure as when more selective sorbents are used and therefore the analyst must exercise care in a choice of conditioning and wash solvents to allow for efficient sample clean up.

In order for SPE to be a success it is useful to follow a systematic approach for method development. A general approach for the preparation and use of C_{18} SPE columns is depicted in Figure 2.3. Most reversed-phase SPE procedures follow a similar scheme as a basis for aqueous sample preparation.

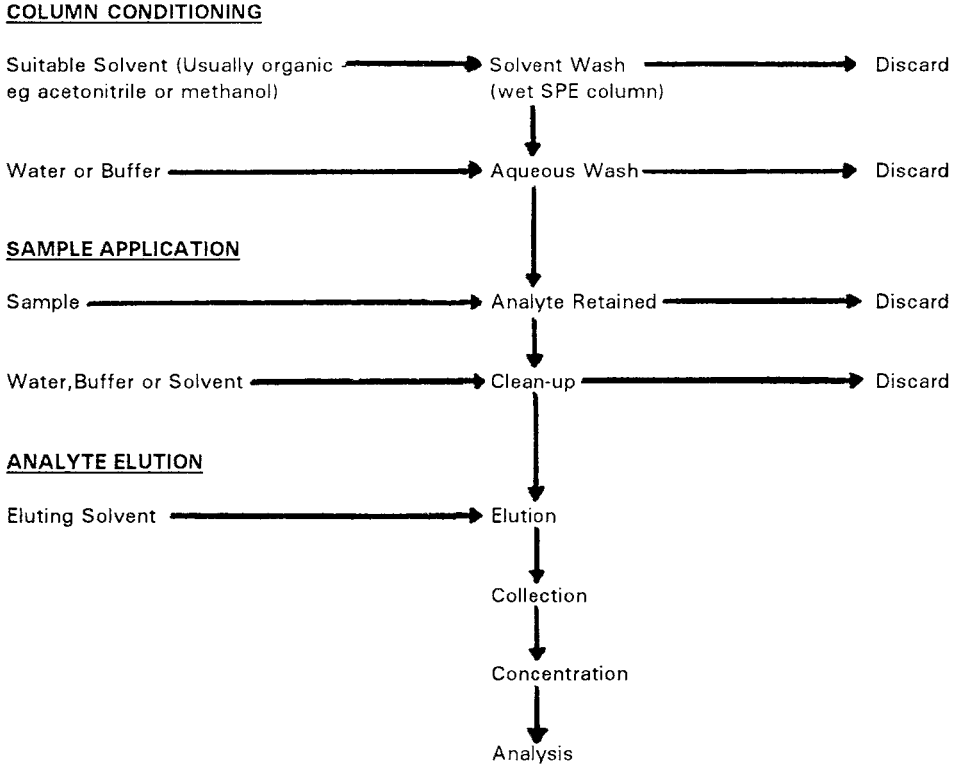


Figure 2.3 Liquid-Solid extraction scheme for the preparation of aqueous samples on non-polar stationary phases. (103).

Using this scheme as an outline and following other procedures developed in our laboratory a SPE procedure was developed for the extraction of cyclizine and its metabolite norcyclizine from biological fluids.

2.2.2.1 Sample Preparation

One millilitre serum samples were added to test tubes and prior to the loading of samples onto the extraction column serum proteins were precipitated by the addition of 1ml of acetonitrile to the sample followed by mixing on a Thermolyne Maxi Mix® vortex mixer (Thermolyne Corp., Dubuque, IA, U.S.A.). Following mixing the samples were centrifuged in a Model MF-C microfiltration centrifuge (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.) at 1600xg for 5 minutes. Dilution of the protein free acetonitrile supernatant with 4ml of water, containing internal standard, reduced the eluotropic strength of the solution thereby minimizing the possible loss of drug and internal standard and improving the precision of the assay. In addition, the precipitation of serum proteins prior to the addition of internal standard solution prevents incomplete denaturation of the drug-protein complex due to an increase in serum volume. The samples were then passed over the previously prepared stationary phase under gravity with the aid of glass reservoirs.

2.2.2.2 Extraction Column Preparation

The hydrophobic nature of C_{18} bonded silica SPE columns preclude their use for analysis of aqueous samples without preparing the stationary phase to accept the sample. The preparation of the column involves wetting the bonded silica with a solvating solvent (eg. acetonitrile) in order to wet and open the hydrocarbon chains and allow the functional groups of the bonded phase to interact with solutions to be applied. This was achieved by allowing 5ml of acetonitrile to pass over the sorbent bed without a vacuum. The next phase of SPE involves removal of the solvating solvent and conditioning of the extraction column in order to create an environment that would facilitate retention of compounds of interest. A conditioning wash of elution solvent (5ml) was introduced. The rationale behind inclusion of this wash involved removal of contaminants that may otherwise interfere with the analysis of samples. The inclusion of this additional wash has been suggested as a way to eliminate interference from contaminants adsorbed onto the column from air (104). Excess acetonitrile had previously been removed by washing columns with 5ml of water (108,109,110). The removal of the conditioning wash was effected by inclusion of a 5ml water wash.

2.2.2.3 Wash Solvent Identification

The necessary requirements for an assay for pharmacokinetic studies include a low detection limit, high precision and accuracy, highly reproducible extraction efficiencies and chromatography free from interfering peaks. SPE methods permit all of these provided that careful selection of solvents or combinations of solvents are entertained. In addition to the compounds of interest being retained

after loading endogenous compounds which may interfere with chromatography may also be adsorbed onto the stationary phase. The analyst must therefore identify a solvent or sequence of solvent washes that will remove possible interfering compounds from the extraction column, but not the compound(s) of interest.

A two wash sequence after loading of the sample was used to selectively remove contaminants from the sample. The washes consisted of a 5ml water wash and then a 5ml acetonitrile-water wash. The water wash removes water soluble or polar compounds retained on the extraction column.

Based on the chromatography of cyclizine on reversed-phase C_{18} analytical columns the use of a combination of acetonitrile-water wash to further clean-up samples was attempted. Combinations of acetonitrile-water did not elute the drug or internal standard from the column and therefore was not expected to remove cyclizine and internal standard from the SPE cartridge.

Various compositions of acetonitrile-water washes were used for the clean-up following the water wash. The wash solutions investigated contained 30%, 40% and 50% water. In each case the columns were washed with 5ml of acetonitrile-water in 1ml aliquots. Each 1ml aliquot was collected and evaporated to dryness using a Model SVC100H rotary vacuum centrifuge connected to a Model RT 100A refrigeration unit (Savant Instruments Inc., Hicksville, NY, U.S.A.). The samples were reconstituted with $100\mu\ell$ of acetonitrile and a $10\mu\ell$ aliquot analyzed. In all cases, despite contamination being present no cyclizine or internal standard was eluted from the SPE column. Reconstitution of samples with $100\mu\ell$ of water also revealed no cyclizine or internal standard in the washes. Of all the wash solvent combinations used, the acetonitrile-water (7:3) was selected as it produced the most contaminated washings indicating that extensive clean-up of the extraction cartridge had occurred.

2.2.2.4 **Elution Solvent Determination**

The establishment of a suitable wash sequence to effect sample clean-up in no way guarantees absolute cleanliness of the extract. It is therefore important to selectively remove the compounds of interest from the extraction column with a suitable solvent or solvent combination in as small a volume as possible.

In order to determine a suitable elution solvent a 1ml sample of serum was extracted using the previously described column preparation. In all cases elution was performed using $500\mu\ell$ aliquots of solvent combinations. Each aliquot was collected, evaporated to dryness, reconstituted with $100\mu\ell$

of water and a 10$\mu\ell$ aliquot analyzed by HPLC. The results of these investigations are depicted in Table 2.1.

Table 2.1 Elution Profile of Cyclizine with Various Elution Solvents.

Composition of Elution Mixture*		Aliquot of Elution Mixture 1	2	3	4	5	6
	Aliquot Volume	500$\mu\ell$	500$\mu\ell$	500$\mu\ell$	500$\mu\ell$	500$\mu\ell$	500$\mu\ell$
40:60**	Peak Height of Cyclizine (mm) in Aliquots of Elution Mixture	0	19	52	47	13	0
60:40♦		64	97	11	4	0	0
60:40□		62	64	35	5	0	0
70:30○		75	42	23	0	0	0
80:20◊		74	50	19	0	0	0

* Acetonitrile-phosphate buffer (0.05M, pH=3)
** Mobile phase (§ 2.1.6)
♦ Preceded by acetonitrile-water (50:50) wash
□ Preceded by acetonitrile-water (60:40) wash
○ Preceded by acetonitrile-water (70:30) wash
◊ Preceded by acetonitrile-water (80:20) wash

Initial investigations involved the use of mobile phase as an elution solvent. The results depicted in Table 2.1 show that 3ml of mobile phase was necessary to elute all the cyclizine from the extraction column. The volume of aqueous component in this elution mixture is approximately 2ml which would prolong any concentration step. In addition, the resulting chromatograms showed contamination that interfered with the cyclizine peak indicating that non-selective elution of the drug had occurred.

In order to elute the drug with a smaller volume of elution solvent more acetonitrile was added. The samples were prepared in the manner described previously but the penultimate wash was an acetonitrile-water (50:50) wash. All the drug was eluted with 2ml of the elution mixture but the resulting chromatograms still showed a contaminating peak at a retention time similar to that of cyclizine. The penultimate wash was changed to an acetonitrile-water combination of the same proportions as the elution mixture. However, this change did not reduce the amount of elution mixture required to elute all the drug and did not improve the cleanliness of the extract significantly.

Subsequently additional acetonitrile was added to the elution mixture and an elution solvent of acetonitrile-phosphate buffer (70:30) was found to elute all of the cyclizine from the extraction column in 1.5ml. The penultimate wash was changed to an acetonitrile-water mixture in the same proportions as the elution solvent. The contamination that had been present when using elution

solvents with more water was significantly reduced. However, there was evidence of a small amount of contaminant that could interfere with the quantitative analysis of cyclizine.

Increasing the proportion of the acetonitrile component (80:20) of the elution mixture did not further reduce the amount of solvent required to elute cyclizine and produced significantly more contaminants in the chromatograms. Therefore elution of the drug was achieved by using three 500$\mu\ell$ aliquots of acetonitrile-phosphate buffer (70:30).

2.2.2.5 Sample Concentration and Reconstitution

Following therapeutic doses of the antihistaminic type compounds blood levels in the ng/ml range would ordinarily be expected. Since the final elution volume is larger than the sample volume, concentration of the sample prior to injection onto the chromatographic system is necessary. The eluate was therefore evaporated to dryness at 40°C in a rotary vacuum centrifuge (Savant Instruments Inc., Hicksville, NY, U.S.A.). The resultant dry residue consisted of buffer salts and analyte. Initially reconstitution was attempted with 100$\mu\ell$ of HPLC grade water. Resultant chromatograms all displayed a contaminant that would interfere to some degree with the cyclizine peak. The contamination was possibly due to injection of a high concentration of buffer. Attempts to effect reconstitution with 100$\mu\ell$ mobile phase were more successful but in several samples long solvent fronts and contaminating peaks interfered with quantitation of the drug. Mathies and Austin (113) have reported a unique method of sample preparation for analgesics in which the addition of acetonitrile and a mixture of salts are added to serum samples and a phase separation effected. The analysis of macrolide antibiotics in our laboratory by SPE also utilized a phase separation, but as the final step of the extraction procedure (108,109,110). The micro-phase separation is effected by the addition of water and acetonitrile to the dry eluate residue and relies on the concentrated buffer solution to force the separation of the acetonitrile phase and allows partitioning of drug into that phase. It is obvious that volumes of the respective phases are critical and if too much water is added, the salt solution is diluted and a homogenous solution formed, resulting in problems with the cleanliness of the extract. Reconstitution with 20$\mu\ell$ water and 30$\mu\ell$ acetonitrile proved effective in salting-out of the organic layer and partitioning the analytes into the acetonitrile layer. Injection of the upper layer yielded chromatograms that were free from contaminating peaks and sufficiently concentrated to determine the low levels of cyclizine that were expected following administration of the drug to human volunteers. Essentially, the phase separation serves as a concentration and purification procedure. Attempts to improve the phase separation with the addition of buffer to the eluate were of little benefit, showing little or no improvement in the cleanliness of the extract.

2.2.2.6 Internal Standard

The internal standard solution, containing protryptiline hydrochloride (1ng/ml) was prepared by dissolving 10mg of protryptiline hydrochloride in 100ml of water in an A grade volumetric flask. A one millilitre aliquot of this solution was removed by pipette and transferred to a 100ml volumetric flask and made up to volume with water.

2.2.2.7 Serum Extraction Method

A 1ml sample of serum was deproteinized by mixing with 1ml of acetonitrile, vortexing for 30 seconds and then centrifuging for 5 minutes at 1600xg. The resultant supernatant was transferred to a 15ml culture tube (Kimble, Vineland, NJ, U.S.A.) containing 4ml of water and 100ng of protryptiline hydrochloride that had been added as a solution (100$\mu\ell$) with the aid of a Micro Lab®P automated dispenser (Hamilton Bonaduz AG, Bonaduz, Switzerland). The diluted supernatant was vortexed for 30 seconds and loaded onto a pre-washed 1ml Bond Elut® C_{18} disposable extraction column (Analytichem International Inc., Harbor City, CA, U.S.A.) with the aid of a custom-made glass reservoir. The pre-wash sequence involved wetting the column with 5ml acetonitrile and then washing with 5ml acetonitrile-0.05M (pH=3) phosphate buffer (70:30) followed by a 5ml water wash. On completion of sample loading the columns were washed with 5ml water followed by a 5ml acetonitrile-water wash (70:30). Washing, sample loading and clean-up of the columns and sample were achieved by allowing the respective solutions to drain through the columns under gravity. Care was taken to ensure that drying out of the column did not occur at any stage of sample preparation. Drying of the column during extraction would necessitate re-wetting of the stationary phase prior to continuing sample preparation. Following the final wash, columns were dried under vacuum using a modified Vac-Elut® system (Analytichem International Inc., Harbor City, CA, U.S.A.). Cyclizine and internal standard were eluted into 3ml Kimax tapered collection tubes (Kimble, Vineland, NJ, U.S.A.), with three 500$\mu\ell$ aliquots of acetonitrile-0.05M (pH=3) phosphate buffer (70:30). The samples were then evaporated to dryness at 40°C in a rotary vacuum centrifuge (Savant Instruments Inc., Hicksville, NY, U.S.A.). The residue was reconstituted in 20$\mu\ell$ of water and vortexed for 1 minute. On addition of 30$\mu\ell$ acetonitrile two layers formed. The samples were then vortexed for a further minute and centrifuged at 1600xg for 30 seconds. All samples were kept sealed to prevent excessive loss of acetonitrile. An aliquot (25-30$\mu\ell$) of clean supernatant was transferred to a WISP limited-volume insert (Waters Assoc., Milford, MA, U.S.A.) using a 100$\mu\ell$ microsyringe (Hamilton Company, Reno, NA, U.S.A.). Aliquots (2-10$\mu\ell$) of the supernatant were injected onto the column for analysis. Chromatograms of samples prepared using this method are depicted in Figure 2.4.

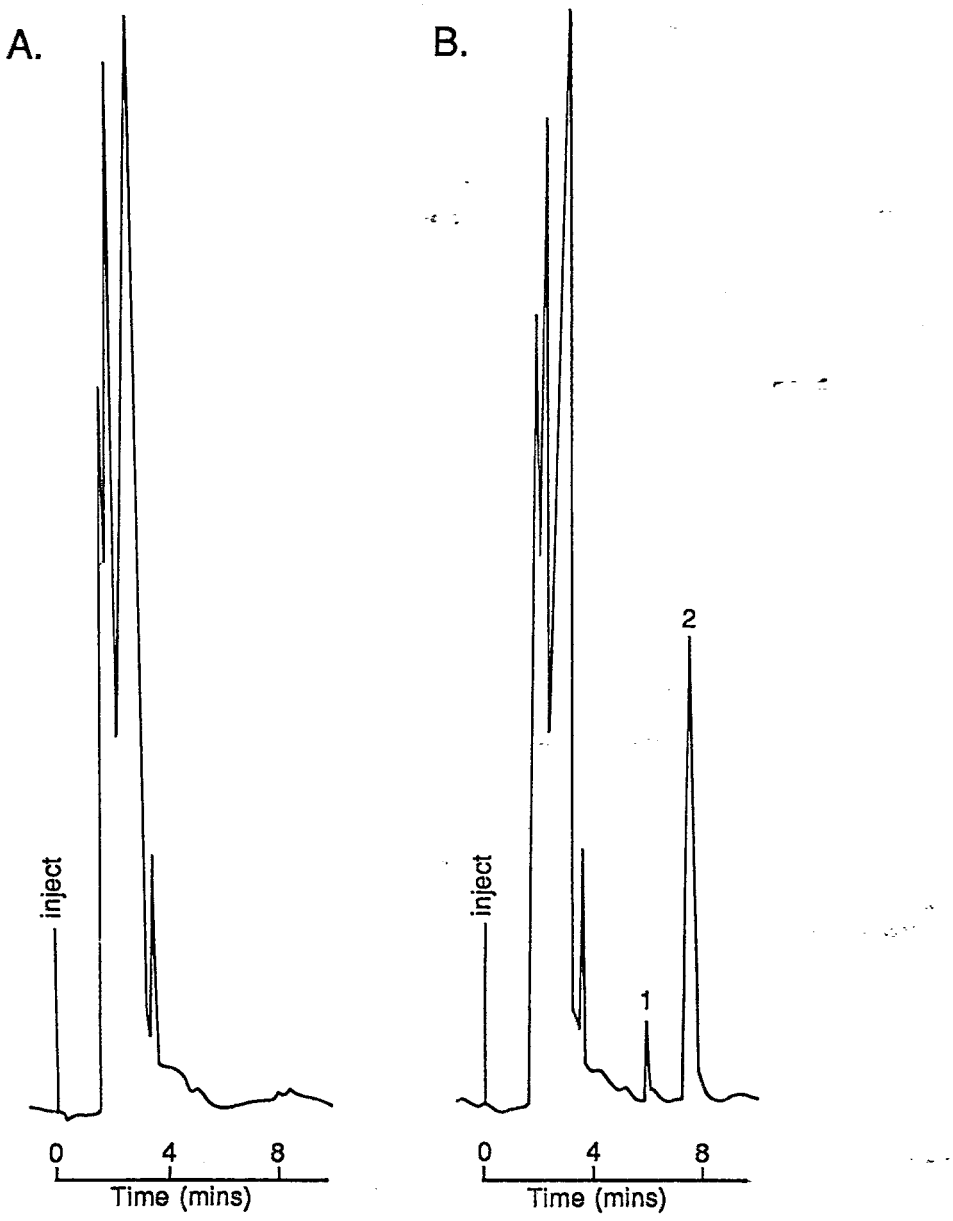


Figure 2.4 HPLC chromatograms of (A) blank human serum, and (B) human serum spiked with 5ng/ml of cyclizine (1) and 100ng/ml of internal standard (2).

2.2.2.8 Identification and Elimination of Sources of Contamination

A source of frustration for an analyst developing a method for the detection of trace quantities of drug in biological fluids is the presence of unwanted contaminants. Of particular importance is possible contamination due to extensive use of plastics in laboratory procedures. Junk *et al* (120) undertook an extensive investigation of the source of spurious peaks during the analysis of pesticides by GC and SPE. These were attributed to plasticizers, antioxidants and other materials possibly present in SPE cartridges. Similarly, commercially available vacuum manifolds used to aid elution from SPE cartridges, consist of plastic type materials and have been implicated as a possible source of contamination (115). It was found that various compounds were eluted from the manifold by the

elution mixture and these interfered with the quantitative analysis of josamycin. The vacuum manifold was modified by fitting stainless steel luer needles through the plastic top of the manifold eliminating contact of the elution mixture with the plastic thereby eliminating contamination. This modified manifold was used in the elution step of the cyclizine extraction.

2.2.3 Validation of the Analytical Method - Serum Extraction

Prior to application for pharmacokinetic studies it is necessary to evaluate the analytical method with respect to the well-established performance criteria of precision, accuracy, sensitivity, specificity, linearity and extraction efficiency or recovery. It is prudent to discuss each term prior to embarking on a discussion of the validation procedure.

Accuracy and precision are terms that are usually discussed together without explanation of either. The determination of accuracy of an assay procedure has been the subject for much debate and has prompted a recent report on bioanalytical method validation in which the requirements for accuracy and precision are discussed (121). Accuracy has been defined by the International Standards Organization (ISO) as the closeness of the measured value or test result to the true or reference value and precision as the variation within replicate measurements or closeness of independent test results obtained under prescribed conditions (121,122). Accuracy has been assessed by interpolation of calibration standard ratios from the regression equation derived from the calibration data and the results reported as a comparison of "amount found" and "amount added" and the measure of this difference reported as the bias (124). Alternatively, accuracy samples can be prepared by an independent analyst and these samples are analyzed "blind" by the analyst. This is in effect the construction of an additional calibration line and there is no guarantee that the analyst making up the standards is any more skilled than the analyst preparing his own calibration standards (111). Peng and Chiou (123) have stated that accuracy should be reported as a percent difference between the mean values of calibration samples and the true or known concentrations. Linearity is usually investigated as a function of the concentration range that is expected and accuracy and precision of data are dependent on it (123). The sensitivity or limit of quantitation (LOQ) of the assay is a statistically validated concentration below which no data are valid or reported (124). Therefore, LOQ is defined as the nominal concentration at which the coefficient of variation (CV) exceeds 15 to 20 percent (125). The specificity of the assay must be demonstrated by the ability of the analytical method to determine only the compound(s) of interest and remain unaffected by the presence of other substances (111). The recovery of the analytical method has been defined as the detector response to pure standards compared to the equivalent amounts recovered from biological specimens (124).

2.2.3.1 Calibration and Linearity

Calibration is undoubtedly one of the most important steps of chemical and drug analysis and good calibration procedures are necessary to ensure that precision and accuracy are obtained (123). Linearity was established by the preparation and extraction of calibration standards prepared using drug-free serum spiked with known quantities of cyclizine. Norcyclizine was not included in these standards as previously published methods indicated that the demethylated metabolite was likely to be present in quantities below the LOQ (46,60). Calibration standards over the concentration range 0-100ng/ml were prepared by dilution of a serum stock solution. An aqueous solution of cyclizine hydrochloride was prepared by accurately weighing 10mg of cyclizine hydrochloride into a 100ml A grade volumetric flask and making up to volume with water. To ensure that complete solution had occurred, the solution was placed in a Model 8845-30 ultrasonic bath (Cole-Parmer Instrument Comp., Chicago, IL, U.S.A.) for 5 minutes (solution A). A 1ml aliquot of solution A was then diluted to 10ml with water yielding a solution of 10μg/ml of cyclizine hydrochloride (solution B). The upper standard (100ng/ml) was prepared by pipetting 1ml of solution B into a 100ml volumetric flask and making up to volume with blank serum. The desired standards (70, 50, 20, 10 and 5ng/ml) were prepared by dilution of the upper standard.

2.2.3.2 Precision and Accuracy

Within run precision of the assay was determined by calculating the coefficient of variation or relative standard deviation of peak height ratios of calibration standards (n=5). Intermediate or between day precision has been defined by the International Standards Organization (ISO) and is a practical alternative to determining assay reproducibility (the closeness in agreement between individual test results obtained with the same method on identical test material but in different laboratories, with different operators using different equipment). Hartmann *et al* (121) have recently commented on the ISO definitions for repeatability and reproducibility and the ISO definition of intermediate precision. Intermediate precision is a recently defined requirement for method validation and was not assessed for this assay. Accuracy was assessed by the interpolation of peak height ratios of calibration standards from the regression equation derived from those standards.

2.2.3.3 Extraction Efficiency

Extraction efficiency or percentage recovery from spiked samples was determined by comparison of peak height ratios of aqueous reference samples and samples to which internal standard was added after completion of the extraction. Spiked serum samples (10 and 100ng/ml) were prepared as

standard were evaporated to dryness, reconstituted and a 30$\mu\ell$ aliquot injected onto the chromatograph. A comparison of the peak height ratio of cyclizine to internal standard in the samples versus reference standards allowed the determination of percentage recovery. The removal of the acetonitrile layer following phase-separation and evaporation was essential to provide a known reconstitution volume enabling accurate comparison of samples and standards. This is necessary since there is a degree of miscibility of the acetonitrile and buffer reducing the volume of acetonitrile.

2.2.3.4 **Limit of Quantitation (LOQ)**

One of the most important considerations for pharmacokinetic studies is the precise measurement of low concentrations of drug and thus the limit of detection (LOD) of a method is of prime importance (111). The use of the term "sensitivity" to define the parameter, LOD, is fairly common, but taken in context refers to the slope of the concentration-response line and therefore is a measure of the degree of sensitivity of the end point to changing concentration. Chamberlain (111) defines the LOD of a method as the smallest single result that can be distinguished from a blank sample with 95% probability. If no blank sample exists then the limit of detection is defined as the lowest concentration at which the estimated precision becomes greater than a given limit. Peng *et al* (123) suggest that the LOD (based on a signal to noise ratio of 3) is not as relevant to describing a method as the limit of quantitation (LOQ) (based on a higher signal to noise ratio). The LOQ as defined by Ludden (125) is the lowest concentration of drug that can be assayed in a biological sample with the desired level of precision and accuracy and is the preferred standard for method validation.

2.2.4 <u>Results and Discussion</u>

2.2.4.1 **Calibration and Linearity**

A calibration curve was constructed by plotting (Figure 2.5) the peak height ratios of cyclizine to internal standard versus cyclizine concentration and performing least squares linear regression. The results of this analysis are depicted in Table 2.2. The calibration curve had a slope of 0.0206 and a y-intercept of 0.10198 with a correlation coefficient of 0.9981.

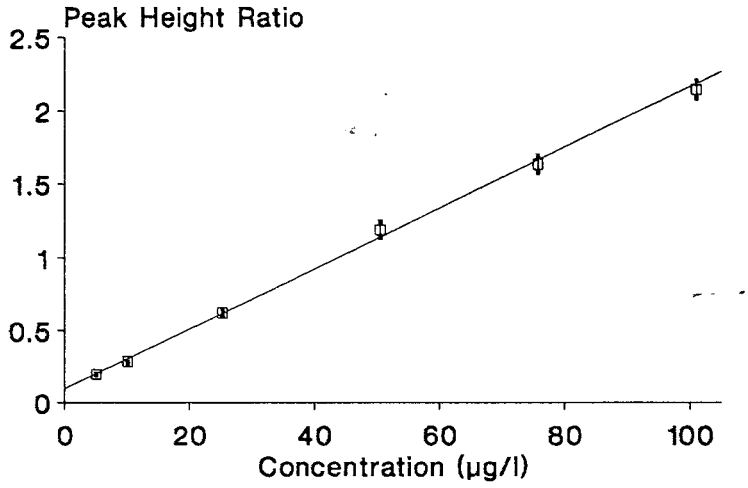


Figure 2.5 Calibration curve constructed after linear regression of mean peak height ratios versus concentration after the extraction of replicate samples. Linear regression equation:- y=0.0206x + 0.1019.

2.2.4.2 Precision and Accuracy

Results from the precision and accuracy studies are tabulated in Table 2.2. Accuracy data are recorded as the determined concentration of each calibration standard. In all but one case percent bias or accuracy was ≤ 10 percent. In all cases relative standard deviations were less than 6 percent. Peng and Chiou (123) consider a relative standard deviation and bias of ≤ 10% to be acceptable for pharmacokinetic studies. In accordance with these criteria this assay is thus highly acceptable for pharmacokinetic studies.

Table 2.2 Precision and Accuracy Results for Extraction of Cyclizine from Serum using the Method Described in § 2.2.2.7.

Calibration and Precision Data				Accuracy Data		
*CYC (ng/ml)	**Base (ng/ml)	***(PHR) (n=5)	R.S.D. %	CYC Found ± S.D. (ng/ml)	Base Found (ng/ml)	% Bias
5.05	4.44	0.1983	5.1	4.68 ± 0.24	4.12	- 7.21
10.10	8.88	0.2780	3.6	8.55 ± 0.31	7.52	- 15.32
25.25	22.21	0.6235	4.0	25.32 ± 1.01	22.27	+ 0.27
50.50	44.42	1.1892	4.8	52.78 ± 2.90	46.43	+ 4.33
75.75	66.63	1.6315	4.1	74.25 ± 3.07	65.31	- 1.98
101.00	88.84	2.1763	1.0	100.70 ± 0.98	88.58	- 0.29

* Cyclizine hydrochloride concentration
** Cyclizine base equivalent concentration
*** Peak height ratio of cyclizine/internal standard

2.2.4.3 Extraction Efficiency

Results from extraction efficiency studies are tabulated in Table 2.3. The mean recovery value obtained for cyclizine from serum was 83%. The loss of 17% can probably be accounted for by loss during the wash sequence after loading of samples onto the extraction column as well as the failure of cyclizine to partition into the acetonitrile layer thus remaining in the aqueous layer during the phase-separation step of reconstitution. In addition, incomplete recovery of all the acetonitrile phase during the phase-separation step would result in a lower extraction efficiency

Table 2.3 Extraction Efficiency Data after the Extraction of Cyclizine from Serum using the Method Described in § 2.2.2.7.

Spiked Sample Concentration (ng/ml)	Aqueous Reference Samples (n=5) *PHR ± R.S.D. %	Extracted Serum Samples (n=4) *PHR ± R.S.D. %	Percentage Recovery
10	0.156 ± 3.5	0.134 ± 3.4	86
100	1.308 ± 3.1	1.046 ± 3.6	80

*Peak height ratio of cyclizine/internal standard

2.2.4.4 Limit of Quantitation (LOQ)

The limit of quantitation based on a relative standard deviation and percentage bias of ≤ 10% was found to be 5ng/ml. This corresponds to an on column load of < 2ng of cyclizine in a 10$\mu\ell$ injection and to 33% of the final amount extracted. The detector sensitivity was 0.01 absorbance units full scale.

2.2.5 Validation of the Analytical Method - Urine Extraction

2.2.5.1 Extraction Procedure

Urine samples were extracted using the method validated for the extraction of cyclizine from serum (§ 2.2.2.7). Urine samples were monitored for both cyclizine and its demethylated metabolite, norcyclizine. Clean extracts were obtained without modification of the extraction procedure. Chromatograms of urine samples prepared using the method described are depicted in Figure 2.6.

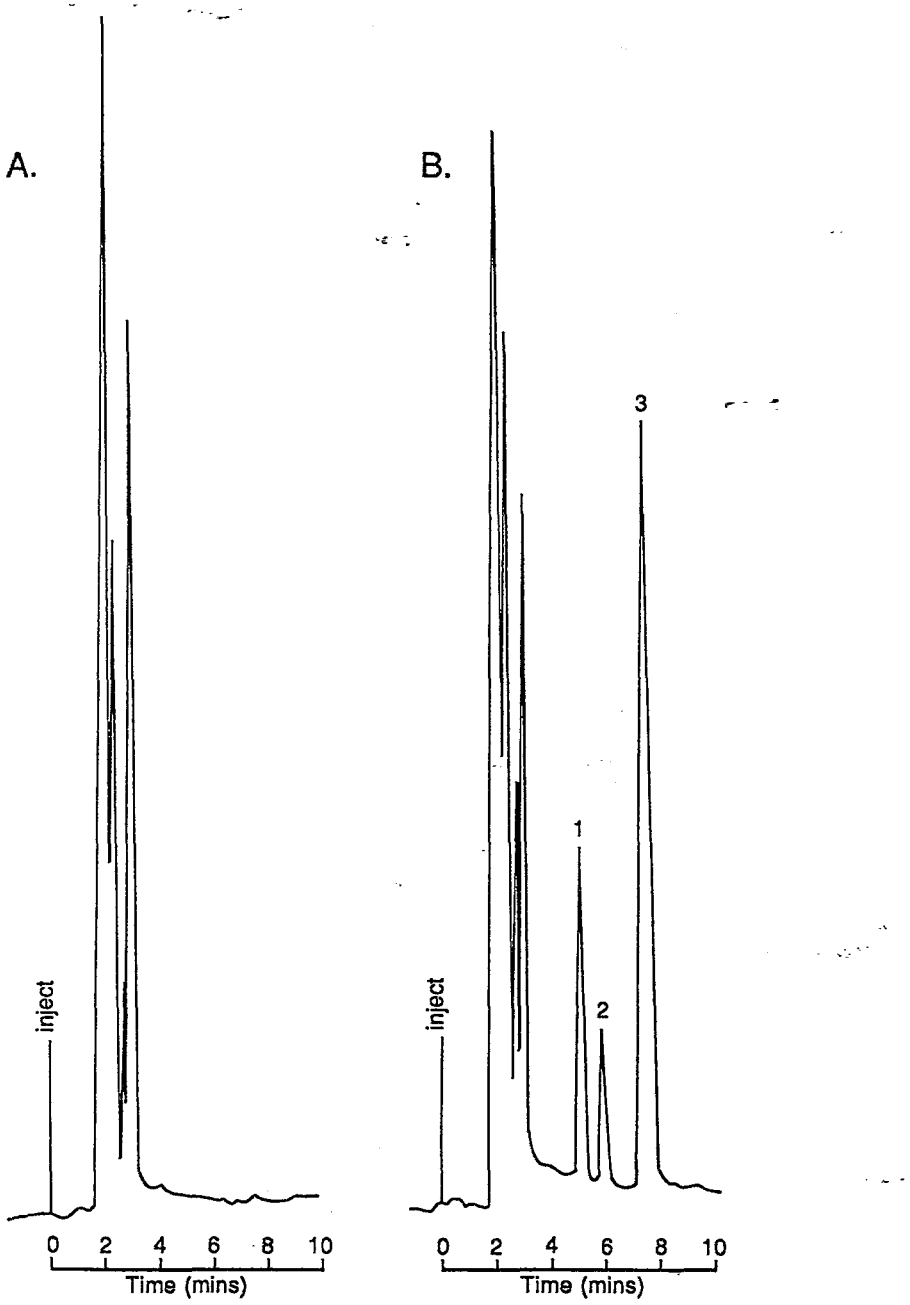


Figure 2.6 HPLC chromatograms of (A) blank human urine and (B) human urine spiked with (1) norcyclizine (50ng/ml), (2) cyclizine (10ng/ml) and (3) internal standard (100ng/ml).

2.2.5.2 Calibration and Linearity

Linearity was established by the preparation and extraction of calibration standards prepared using drug-free urine spiked with known quantities of both cyclizine and norcyclizine. Calibration standards over the concentration range 0-100ng/ml were prepared by dilution of a urine stock solution containing cyclizine and norcyclizine. An aqueous stock solution of cyclizine hydrochloride was prepared by accurately weighing 10mg of cyclizine hydrochloride into a 100ml volumetric flask and making up to volume with water. To ensure complete solution had occurred the solution was placed in a Model 8845-30 ultrasonic bath (Cole-Parmer Instrument Comp., Chicago, IL, U.S.A.) for 5

minutes (solution A). A 1ml aliquot of solution A was then diluted to 10ml of water yielding a solution of 10μg/ml of cyclizine hydrochloride (solution B). A stock solution of norcyclizine was prepared by accurately weighing 10mg of norcyclizine into a 100ml volumetric flask and making up to volume with acetonitrile. The solution was placed in an ultrasonic bath for 5 minutes to ensure that all the norcyclizine had dissolved (solution C). A 1ml aliquot of solution C was diluted with 10ml of water to yield a solution of 10μg/ml of norcyclizine (solution D). The upper standard (100ng/ml) was prepared by pipetting 1ml of solutions B and D into a 100ml volumetric flask and making up to volume with blank urine. The desired standards (75, 50, 25, 10 and 5ng/ml) were prepared by dilution of the upper standard.

2.2.5.3 **Precision and Accuracy**

Within run precision of the assay was determined by calculating the relative standard deviation of peak height ratios of calibration standards (n=5). Accuracy was assessed from peak height ratios of calibration standards from the linear regression equation derived from those standards.

2.2.5.4 **Extraction Efficiency**

The percentage recovery from spiked urine samples was determined as detailed in § 2.2.3.3. Replicate samples of urine stock solutions containing 10ng/ml and 100ng/ml of cyclizine and 10ng/ml and 100ng/ml of norcyclizine were extracted.

2.2.6 <u>Results and Discussion</u>

2.2.6.1 **Calibration and Linearity**

A calibration curve was constructed by plotting the peak height ratios of cyclizine and norcyclizine to internal standard versus norcyclizine (Figure 2.7) or cyclizine (Figure 2.8) concentration and performing least squares linear regression analysis. The results of this analysis are tabulated in Tables 2.4 and 2.5 for cyclizine and norcyclizine respectively. The calibration curves had slopes of 0.00614 and 0.00794, y-intercepts of 0.01608 and 0.01502 and correlation coefficients of 0.9970 and 0.9960 for cyclizine and norcyclizine respectively.

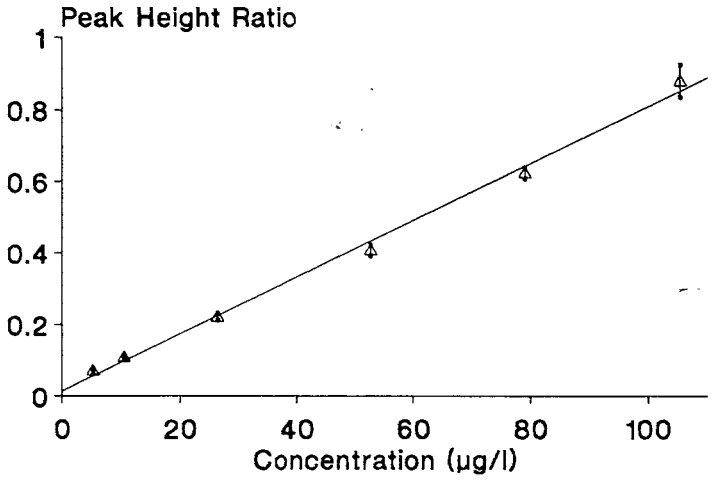


Figure 2.7 Calibration curve constructed after linear regression of mean peak height ratios versus concentration after the extraction of replicate samples of norcyclizine in urine. Linear regression equation:- y=0.00794x + 0.01502.

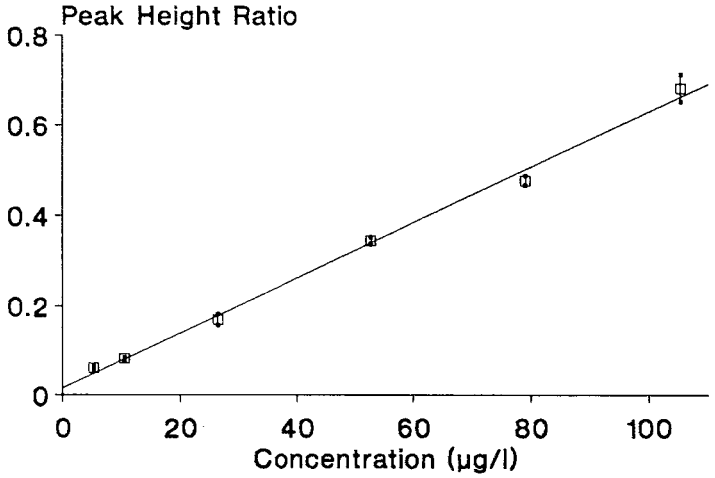


Figure 2.8 Calibration curve constructed after linear regression of mean peak height ratios versus concentration after the extraction of replicate samples of cyclizine in urine. Linear regression equation:- y=0.00614x + 0.01608.

2.2.6.2 Precision and Accuracy

Table 2.4 lists the results of precision and accuracy studies for cyclizine in urine and Table 2.5 those results for norcyclizine in urine. Accuracy data are recorded as the determined concentration of each calibration standard for both cyclizine and norcyclizine. The percent bias was $\leq \pm 10$ percent for all samples save the lowest concentrations for norcyclizine and cyclizine.

Table 2.4 Precision and Accuracy Data for the Extraction of Cyclizine from Urine using the Method Described in § 2.2.5.1.

Calibration and Precision				Accuracy		
*CYC (ng/ml)	**Base (ng/ml)	***PHR (n=5)	R.S.D. %	CYC Found ± S.D. (ng/ml)	Base Found (ng/ml)	% Bias
5.27	4.64	0.05341	4.8	6.08 ± 0.29	5.35	+13.32
10.54	9.27	0.08694	4.2	11.54 ± 0.48	10.15	+8.67
26.35	23.18	0.1739	4.0	25.71 ± 1.04	22.61	-2.43
52.70	46.35	0.3487	2.3	54.17 ± 1.25	47.65	+3.67
79.05	69.53	0.4817	2.2	75.83 ± 1.68	66.70	-4.07
105.40	92.71	0.6632	1.7	105.39 ± 1.75	92.70	-0.0094

* Cyclizine hydrochloride concentration
** Cyclizine base equivalent concentration
*** Peak height ratio of cyclizine/internal standard

Table 2.5 Precision and Accuracy Data for the Extraction of Norcyclizine from Urine using the Method Described in § 2.2.5.1.

Calibration and Precision			Accuracy	
♦NCYC (ng/ml)	♦♦PHR (n=5)	R.S.D. %	NCYC Found ± S.D. (ng/ml)	% Bias
5.27	0.06933	7.9	6.84 ± 0.54	+22.95
10.54	0.1077	4.5	11.67 ± 0.52	+9.68
26.35	0.2197	4.0	25.78 ± 1.04	-2.16
52.70	0.4064	4.2	49.29 ± 2.08	-6.47
79.05	0.6227	2.7	76.54 ± 2.04	-3.18
105.40	0.8798	5.2	108.92 ± 5.68	+3.23

♦ Norcyclizine concentration
♦♦ Peak height ratio of norcyclizine/internal standard

2.2.6.3 **Extraction Efficiency**

The results from percentage recovery studies are tabulated in Table 2.6. The mean recovery value for cyclizine from urine was 84% and that for norcyclizine 83%. Some loss is likely to have occurred during the acetonitrile/water wash and during reconstitution when incomplete partitioning of the cyclizine and norcyclizine into the acetonitrile during the phase-separation step may have occurred.

Table 2.6 Extraction Efficiency Data after the Extraction of both Cyclizine and Norcyclizine from Urine using the Method Described in § 2.2.5.1.

Spiked Sample Concentration (ng/ml)	Aqueous Reference Samples (n=5) *PHR ± R.S.D. %	Extracted Urine Samples (n=4) *PHR ± R.S.D. %	Percentage Recovery
Cyclizine			
10	0.156 ± 3.5	0.139 ± 2.5	89
100	1.308 ± 3.1	1.03 ± 4.7	79
Norcyclizine			
10	0.216 ± 2.2	0.197 ± 1.9	91
100	1.770 ± 1.9	1.264 ± 2.0	75

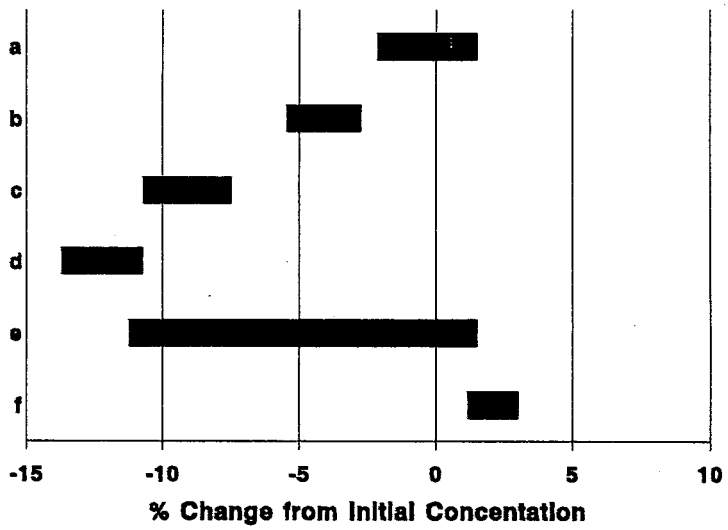
*Peak height ratio of cyclizine or norcyclizine/internal standard

2.3 STABILITY OF CYCLIZINE AND NORCYCLIZINE IN BIOLOGICAL FLUIDS

In order to ensure the collection of reliable pharmacokinetic information about a drug, it is necessary to have a knowledge of long term storage stability of all compounds being analyzed in all biological fluids being assayed. The acquisition of such information is essential as in most cases samples collected during pharmacokinetic studies are not assayed immediately. The length of a stability study is usually determined by initial storage requirements, however, the maximum storage time for samples must be established (126). Mehta (91) suggested that stability studies be performed for at least 6 weeks. Samples in our laboratory are stored at either 4°C or -15°C. Some authors suggest that samples awaiting analysis be stored at -20°C, -70°C or -80°C, in order to overcome instability problems that may arise due to the presence of water in association with macromolecules at higher temperatures or as a result of freezing point depression due to high salt concentration (123,126).

An additional problem is the lack of information in the literature regarding stability study design and interpretation of results from these studies. Stability is often dismissed by a cursory statement mentioning the fact that samples were stable at -15°C for 4 weeks by comparison of samples analyzed on day 1 of the study and comparing concentrations found in stored samples to day 1 results. The use of a simple t-test to distinguish whether a significant difference in concentration between stored and freshly prepared samples has been reported (127,128,129,130). Whilst these authors have attempted to apply specific criteria to stability data only one (130) has specified a specific storage time beyond which samples can be considered not suitable for analysis.

Timm *et al* (131) have developed a systematic procedure for the investigation of the stability of drugs in biological samples. The approach described is based on the statistical treatment of experimental data and takes into account the quality of the experimental procedure (precision of the method, number of replicates). Furthermore, they suggest criteria for the interpretation of stability data based on the construction of a 90% confidence interval within which the true percentage change (Δ) in concentration between freshly prepared and stored samples lies with 90% certainty. The confidence intervals are calculated using the measured percentage difference between freshly prepared and stored samples (D) for replicate samples. In addition, Timm *et al (131)* state that although a statistically significant change may occur it may not be pharmacokinetically relevant. They suggest a degradation of 10% to be of pharmacokinetic importance and suggest criteria to enable a decision on whether a statistically significant change is pharmacokinetically relevant. A decision on sample stability is determined by the position of the calculated 90% confidence interval in relation to true percentage change limits of 0 and -10%. Figure 2.9 depicts the possible results that could be obtained from the analysis of stored samples utilizing this method for data discrimination.



(a) change of response, not significant and not relevant.
(b) decrease of response, significant but not relevant.
(c) decrease of response, significant and possibly relevant.
(d) decrease of response, significant and relevant.
(e) decrease of response, not significant but possibly relevant.
(f) increase of response, significant.

Figure 2.9 Interpretation of stability data as described by Timm *et al* (131).

2.3.1 Experimental Procedure

2.3.1.1 Sample Preparation

Serum and urine samples were prepared from blank serum and urine that had been proven free from contaminants. An aqueous solution of cyclizine hydrochloride was prepared by accurately weighing 10mg of cyclizine hydrochloride into a 100ml volumetric flask and making up to volume with water (solution A). A stock solution of norcyclizine was prepared by accurately weighing 10mg of norcyclizine into a 100ml volumetric flask and making up to volume with acetonitrile (solution B). To ensure complete solution of the cyclizine hydrochloride and norcyclizine, solutions A and B were placed in an ultrasonic bath for 5 minutes. A 1ml aliquot of solution A was diluted to 10ml with water yielding a solution of 10μg/ml of cyclizine hydrochloride (solution C). A 10μg/ml norcyclizine solution was prepared by pipetting 1ml of solution B and making up to 10ml with water (solution D). A serum solution of cyclizine hydrochloride (100ng/ml) was prepared by pipetting 1ml of solution C into a 100ml volumetric flask and making up to volume with blank serum. Thirty five millilitres and five millilitres of this serum solution were diluted to 50ml with blank serum respectively to yield serum solutions containing 70ng/ml (upper concentration) and 10ng/ml (lower concentration) of cyclizine hydrochloride. These solutions were mixed with the aid of a magnetic stirrer Type SS660 (Gallenkamp, England) for 30 minutes. Urine solutions containing cyclizine hydrochloride in concentrations of 70ng/ml and 10ng/ml were prepared in the same way. A urine solution containing norcyclizine (100ng/ml) was prepared by pipetting 1ml of solution D into a 100ml flask and making up to volume with blank urine. Urine solutions containing 70ng/ml and 10ng/ml of norcyclizine were prepared in the same way as the cyclizine solutions. Four 1ml samples of each solution (two serum and four urine) were analyzed immediately as the freshly prepared samples. One millilitre aliqouts of each solution were pipetted into test-tubes for storage at 4°C or -15°C for 1, 2 and 4 weeks.

2.3.1.2 Sample Analysis

Serum and urine samples (n=4) were analyzed as described in § 2.2.2.7. Samples were assayed immediately after preparation (time zero) and then after each of the stated storage intervals. Concentrations of cyclizine and norcyclizine were interpolated from calibration curves generated on each day of analysis with freshly prepared standards (§ 2.2.3.1 and § 2.2.5.2).

2.3.2 Results and Discussion

Confidence intervals (90%) using the measured percentage concentration difference between time zero and stored samples were constructed and are depicted diagrammatically according to the method described by Timm *et al* (131).

2.3.2.1 Serum

The results obtained from stability studies of cyclizine in serum at both the upper and lower concentrations are depicted in Figure 2.10. The 10ng/ml samples stored at 4°C showed a decrease of response that was not significant but possibly relevant at 1 week. This insignificant change of concentration may reflect the imprecision of the assay without being able to exclude possibly relevant instability of the drug. Samples analyzed after 2 and 4 weeks showed changes of response that were not significant and not relevant. However, possibly as a result of assay imprecision, there is an increase in response greater than 10% which may be significant. The upper concentration samples showed no significant or relevant changes at 1 and 2 weeks, but showed a slight increased response at 4 weeks that may be considered significant. The lower concentration samples stored at -15°C show increased responses at 2 and 4 weeks that may be significant. The 70ng/ml samples stored at -15°C show no significant or relevant changes of response. Therefore, serum samples from bioavailability or pharmacokinetic studies may be stored at -15°C for 4 weeks if immediate analysis is not possible.

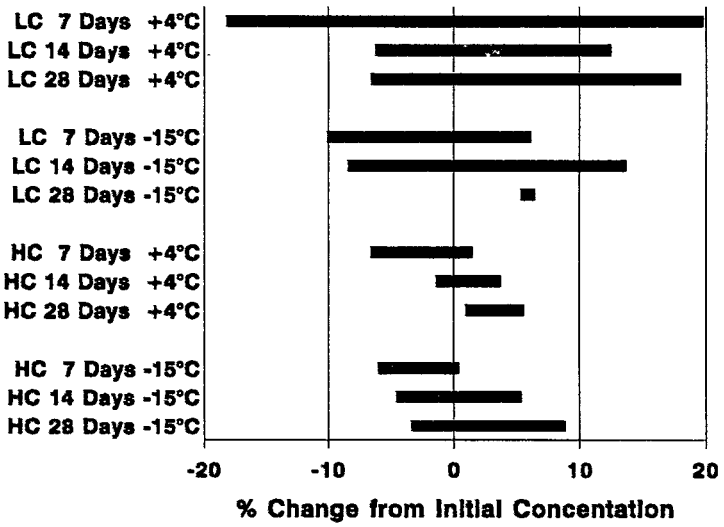


Figure 2.10 Stability of cyclizine hydrochloride in serum at two different concentrations stored at +4°C and -15°C for 1, 2 and 4 weeks.

2.3.2.2 Urine

The results obtained from the stability studies of cyclizine hydrochloride at both the upper and lower concentrations, in urine are depicted in Figure 2.11. The low concentration samples of cyclizine hydrochloride when stored at both 4°C and -15°C showed a decreased response at 4 weeks that was significant and possibly relevant and the upper concentration samples showed either a slight increase, significant but not relevant decrease or a significant increase in response for both storage conditions over the 4 week period. The results for the stability of norcyclizine at both high and low concentrations in urine are depicted in Figure 2.12. Interpretation of the results, particularly at the low concentration, may be unclear as a result of possible imprecision of the analytical method. Nevertheless, it is clear that after 4 weeks at 4°C, the samples showed a significant and relevant decrease in response indicating that use of data from analysis of these samples be interpreted with caution. The upper concentration stored at 4°C also showed a decrease in response after 4 weeks that is not significant but possibly relevant. Once again this result must be treated with caution. The upper concentration samples stored at -15°C appear to be stable for the 4 week period, but low concentration samples showed an increased response that should be considered significant. Further stability studies at this concentration would determine whether the response is real or an artifact of the assay. However, storage of urine samples from bioavailability studies at -15°C for 4 weeks prior to analysis is possible.

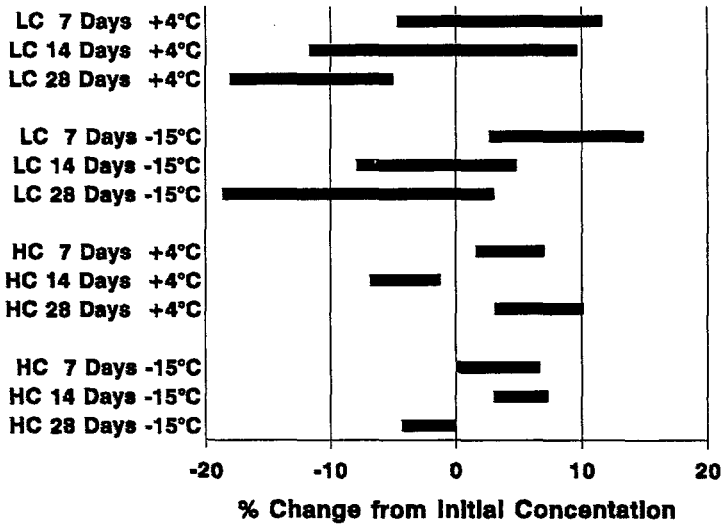


Figure 2.11 Stability of cyclizine hydrochloride in urine at two different concentrations stored at +4°C and -15°C for 1, 2 and 4 weeks.

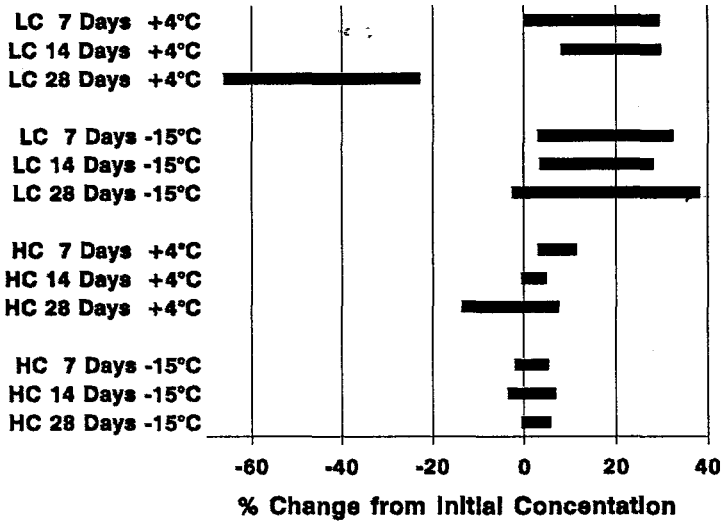


Figure 2.12 Stability of norcyclizine in urine at two different concentrations stored at +4°C and -15°C for 1, 2 and 4 weeks.

2.4 PILOT STUDY

A pilot trial was conducted to generate preliminary pharmacokinetic parameters for cyclizine. A healthy male volunteer, who had fasted overnight, was administered a single oral dose of 50mg cyclizine hydrochloride together with 200ml of water. The volunteer received a light breakfast consisting of toast spread with margarine and jam and a glass of orange juice 2 hours after the dose. Lunch was given 5 hours after the dose.

2.4.1 Serum Collection

Blood samples (10ml) were withdrawn from an arm vein with the aid of an indwelling catheter prior to the dose and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, 9.0, 11.0, 12.0 and 24.0 hours after the dose. The samples were allowed to stand for 30 minutes to allow clotting to occur and then serum was collected after centrifugation of the samples at 1600xg for 25 minutes. The samples were stored at -15°C for two days prior to analysis. Cyclizine was extracted from the serum samples as in § 2.2.2.7 and the samples analyzed using HPLC (§ 2.1.8).

2.4.2 Urine Collection

Urine samples were collected immediately prior to dosing and then at 2.0, 4.0, 6.0 and 8.0 hours after administration of a single 50mg oral dose of cyclizine hydrochloride to a healthy male volunteer. The volume and pH of urine voided during and after each collection period were noted. A 10ml aliquot of urine from each collection period was stored at -15°C for two days prior to analysis by HPLC.

2.4.3 Results and Discussion

2.4.3.1 Serum

Analysis of serum samples revealed the presence of both cyclizine and norcyclizine indicating that the extraction procedure was suitable for the determination of both compounds in serum. Whilst the method allows for the isolation of norcyclizine, the levels of the compound were low and therefore in order to accurately quantitate the levels of norcyclizine, increased sensitivity of the method for detection of norcyclizine is required. The serum concentration versus time profile of cyclizine following administration of a single 50mg cyclizine hydrochloride tablet to a healthy male volunteer is depicted in Figure 2.12. The maximum concentration (C_{max}) of cyclizine obtained and corrected for base equivalent was 49.72ng/ml at a time of 3 hours (t_{max}). The area under the serum concentration versus time curve from 0-24 hours (AUC_{0-24}) was 294.66 ng/ml.hr. The elimination appears biphasic with elimination half-lives of 6 and 22 hours for the early and late phases, respectively. The subject indicated some dryness of mouth 1 hour after the dose and he was drowsy 3 to 5 hours post dosing.

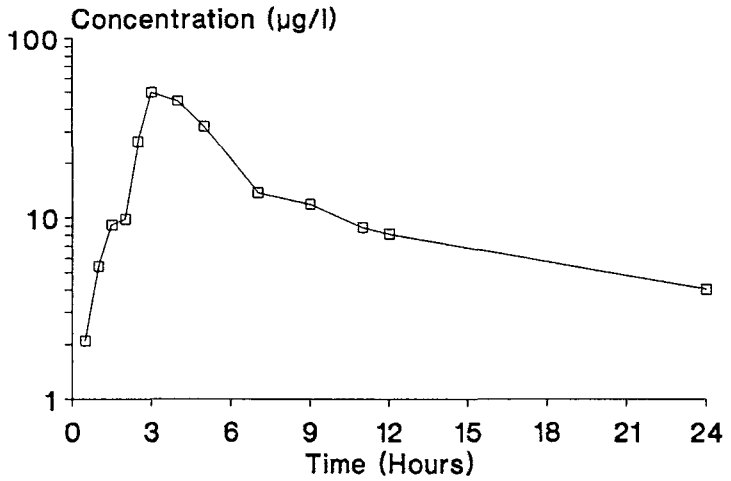


Figure 2.13 Serum concentration-time profile of cyclizine following administration of a single oral dose of 50mg cyclizine hydrochloride to a human volunteer.

2.4.3.2 Urine

Urine samples were analyzed for norcyclizine and cyclizine as in § 2.2.2.7. The concentrations of both cyclizine and norcyclizine were found to be out of the calibration range. This necessitated re-extraction of the samples using 250$\mu\ell$ or 500$\mu\ell$ of urine diluted to 1ml with blank urine. The dilution to 1ml eliminates any possible matrix effects on the extraction process. The results of the 8 hour urine study are tabulated in Table 2.7. The cumulative urinary excretion curves for norcyclizine and cyclizine are depicted in Figure 2.14. The results of this study revealed that up to 8 hours, less than 1 percent of the administered dose is excreted as norcyclizine and less than 1 percent of the dose is excreted as cyclizine. In addition, urine samples should be collected until all the unchanged drug has been excreted (68). The concentrations of both norcyclizine and cyclizine in the urine were approximately ten times higher than previously reported after analysis of samples using a gas-liquid chromatographic method (46).

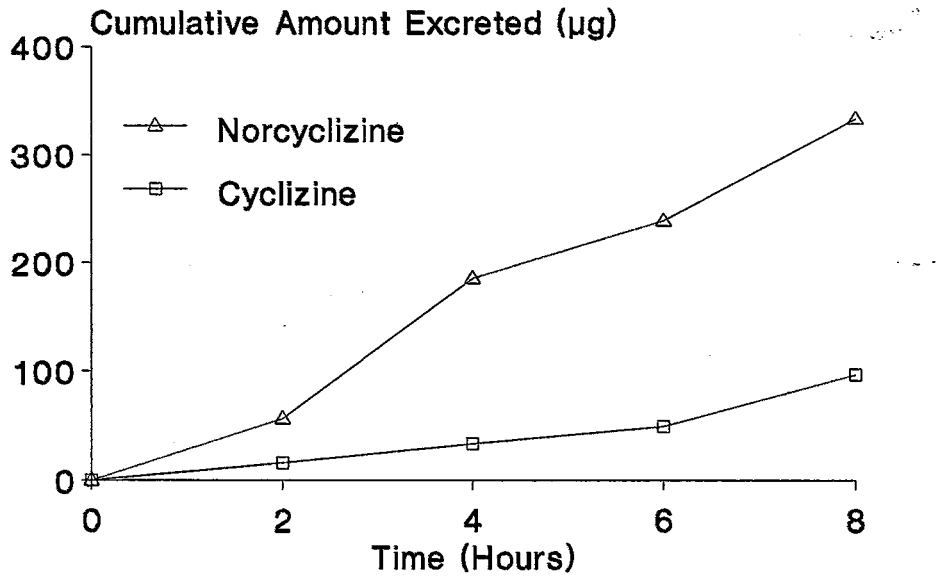


Figure 2.14 Eight hour cumulative urinary excretion curves for both cyclizine and norcyclizine following administration of a single oral dose of cyclizine hydrochloride (50mg) to a healthy male volunteer.

Table 2.7 Eight Hour Urinary Excretion Data Following the Administration of a Single 50mg Cyclizine Hydrochloride Tablet to a Healthy Male Volunteer.

Sample Time (Hours)	pH	*NCYC (ng/ml)	**CYC (ng/ml)	Volume (ml)	Amt. NCYC (μg)	Amt. CYC (μg)
0	6.59	0	0	213	0	0
0 - 2	6.34	246.70	69.10	230	56.74	15.89
2 - 4	6.85	524.78	73.04	245	128.57	17.89
4 - 6	6.60	367.04	108.61	145	53.22	15.75
6 - 8	6.65	420.56	209.33	225	94.63	47.10

* Concentration of norcyclizine
** Concentration of cyclizine (base equivalent)

CHAPTER 3

IMPROVED ANALYSIS OF CYCLIZINE AND NORCYCLIZINE

Analysts often desire improved sensitivity to enhance the credibility of data obtained from pharmacokinetic studies. The previously reported HPLC method (47), for the determination of cyclizine and norcyclizine in biological fluids, has shown that norcyclizine can be analyzed in serum (§ 2.4.3.1). In addition, some samples revealed cyclizine concentrations below 5ng/ml. This necessitated the development of a more sensitive and selective analytical procedure for the determination of cyclizine and its demethylated metabolite, norcyclizine in serum.

Organic molecules, including drugs can be electrochemically oxidised or reduced and amplification of the current generated by these processes can produce a suitable signal (132). The use of liquid chromatography with electrochemical detection (ECD) provides the analyst with a tool for improving the sensitivity of a method with detection limits at the 0.1pmol level being achieved for a number of oxidizable compounds (133,134). However, statements regarding minimum detectable quantities should be taken in context as detector performance in liquid chromatography is dependent on the analyte and chromatographic conditions. In general, electrochemical detectors produce high sensitivities (132). In addition, the analyst is able to "tune" the detector by varying the electrode potential thereby selectively monitoring the analyte of interest without interference from co-eluting substances (133,134). Furthermore, compounds that can only be detected with difficulty at low UV wavelengths may be easily detected with ECD (e.g., the mercaptans and some heterocyclic compounds) (90).

Electrochemical detectors used in conjunction with HPLC fall into one of two categories based on the analytical cell design (i.e., amperometric or coulometric). Amperometric detectors have a low electrolytic efficiency (1-10%) due to the small surface area of the electrode, but have a sensitivity that may be high (92,135). Increasing the surface area of the electrode as in coulometric detectors, may produce 100% electrolysis, at which point the coulometric limit is reached with maximum sensitivity (92,135). Detectors are available as single or dual-electrode detectors (135). One particular advantage of dual-electrodes in series is that high selectivity is possible by pre-oxidation or reduction of interfering compounds at a lower potential at the first electrode and subsequent oxidation or reduction of the compound(s) of interest at a higher potential at the second electrode (135).

Despite the possible advantages of ECD with respect to both sensitivity and selectivity, the use of these detectors are not without drawbacks (132). The detection process requires that the mobile phase be conducting, it must contain some aqueous component and it should be completely free from oxygen (136). If buffered solutions are essential for separation they should be in the low ionic strength range (0.01-0.1M) so as to optimize conductivity with a minimum contribution from impurities from the buffer reagents (133). In addition, high potentials tend to be less selective than low potentials, because more substances are oxidizable at these voltages (137), necessitating extensive sample preparation. Furthermore, the choice of reagents becomes critical, as most solvents available for HPLC are spectroscopically pure, but not electrochemically pure (138), and are an added source of possible baseline abberations. Other areas of concern for the analyst utilizing ECD are disturbances in the electrical power supply, static electricity build-up and discharge, electromagnetic interferences and radio-frequency interference (137). Kissinger (133) has suggested that the entire chromatograph be considered part of the electrochemical cell when utilizing ECD with HPLC.

The success achieved by the use of coulometric detection for the analysis of erythromycin in biological fluids (139,140,141), in our laboratory, suggested that increased sensitivity and selectivity may be possible. Therefore, the determination of low levels of cyclizine and norcyclizine in biological fluids after both intravenous and oral administration of the drug to human volunteers could be feasible using this method of detection.

3.1 ANALYTICAL METHOD DEVELOPMENT

The HPLC system (§ 2.1.3) utilized for the analysis of cyclizine and norcyclizine in biological fluids appeared to be suitable for use with electrochemical detection. Critical aspects of the chromatographic system that are required for HPLC-ECD are satisfied by the use of on-line degassing, constant fluid flow and maintenance of constant temperature. Some modifications of the system were necessary to produce a sensitive, selective and reliable chromatography.

3.1.1 Analytical Column Selection

The "life-span" of an analytical column is highly dependent upon the mobile-phase used, sample clean-up, column storage and the number of samples analyzed. One author (135) has suggested that 1000 samples can be analyzed on a column. The deterioration of an analytical column may be highlighted by one of the following events:- changing retention times, incomplete resolution, split peaks and changes in peak shape. The analytical column previously used for the analysis of cyclizine

(10μm C_{18} column) deteriorated to an extent where it was unusable. Attempts to pack a 10μm C_{18} column that produced similar resolution, retention times and peak shapes failed. Several commercially prepared columns also yielded poor results. Columns prepared with alternative stationary phases were tested and the column that resolved the norcyclizine and cyclizine peaks adequately with a suitable retention time was a 15cm x 4.1mm i.d. stainless steel column packed with Techsil 5μm octadecylsilane (C_{18}) material (HPLC Technology, Wilmslow, United Kingdom). The number of theoretical plates of this column calculated using equation 2.1 was over 17000 indicating a high efficiency column had been prepared.

3.1.2 HPLC Apparatus

The modular chromatographic system consisted of a Waters Model M 6000A dual piston constant flow pump (Waters Assoc., Milford, MA, U.S.A.), an automated Waters Intelligent Sample Processor Model 710B (Waters Assoc., Milford, MA, U.S.A.), a Model 5100A Coulochem dual electrode electrochemical detector with a Model 5010 analytical cell (Environmental Sciences Associates Inc., Bedford, MA, U.S.A.) preceded by a carbon filter and a dual-pen Model 561 strip chart recorder (Hitachi Ltd., Tokyo, Japan) was used to record data. The mobile-phase was constantly degassed using an in-line degasser Model ERC-3510 (Erma Optical Works Ltd., Tokyo, Japan). In addition, the system included a Model 5020 guard cell (Environmental Sciences Associates Inc., Bedford, MA, U.S.A.) preceded by a carbon filter. The analytical column was a custom made 15cm x 4.1mm i.d. stainless-steel column, packed with Techsil 5μm octadecylsilane (C_{18}) material (HPLC Technology, Wilmslow, United Kingdom). The analytical column was preceded by an Uptight Precolumn Kit (Upchurch Scientific Inc., Oak Harbor, WA, U.S.A.) packed with glass beads. Both the guard and analytical columns were maintained at 30°C with a Model LC-22 temperature controller (Bioanalytical Systems INC., West Lafayette, IN, U.S.A.).

3.1.3 Chemicals

All chemicals were at least of analytical reagent grade. HPLC grade solvents were used since electrochemical-grade HPLC solvents are not available. Acetonitrile was distilled-in-glass UV grade (Burdick and Jackson, Muskegon, MI, U.S.A.). AnalaR® phosphoric acid (85%) and sodium hydroxide pellets were obtained from BDH Chemicals Ltd. (Poole, England). Cyclizine base and cyclizine hydrochloride were obtained from Wellcome (Pty) Limited (Johannesburg, South Africa). Norcyclizine (an inactive metabolite of cyclizine) and chlorcyclizine hydrochloride were obtained from the Wellcome Foundation Limited (Dartford, United Kingdom). Protryptiline hydrochloride was obtained from MSD Laboratories (Johannesburg, South Africa). Water used for buffer preparation,

extraction and chromatography was initially prepared by a reverse-osmosis Milli-RO 15 Water Purification System (Millipore, Bedford, MA, U.S.A.) that consisted of a Super-C carbon cartridge, two Ion-X ion exchange cartridges and an Organex-Q cartridge (§ 2.1.4) and filtered with a 0.22μm filter, prior to use.

3.1.4 Mobile-Phase Selection

The selection of a suitable mobile-phase for the newly packed analytical column was performed on the HPLC apparatus detailed in § 2.1.3. Suitable resolution and reasonable retention times were obtained using a mobile-phase of acetonitrile-phosphate buffer 0.05M pH=3 (3:7). The mobile-phase buffer was prepared as discussed in § 2.1.6. Following mixing of the acetonitrile and buffer, the mobile-phase was degassed, under vacuum, and filtered through a 0.45μm HVLP Millipore filter prior to use.

3.1.4.1 Mobile-Phase Considerations in Electrochemical Detection

The manufacturers of the Model 5100A Coulometric detector suggest several methods of improving sensitivity and selectivity with respect to mobile-phases for electrochemical detection (138). The use of carbon prefilters is advised to prevent unnecessary exposure of both the guard and the analytical cell electrodes to particles that may be present in the mobile-phase. On-line degassing is useful for preventing dissolved gases in the mobile-phase interfering with chromatograph stability. Recirculation of mobile-phase (if a large volume is used) in a system with a guard cell is not only economical, but decreases background current and subsequent noise due to removal of oxidizable faradic impurities that may be present in the mobile-phase.

3.1.5 Electrochemical Detection of Cyclizine, Norcyclizine and Internal Standard

In order to determine the optimum potential for the determination of a compound it is necessary to generate a voltammogram. The generation of a voltammogram may be accomplished by one of two methods, namely, cyclic voltammetry or hydrodynamic voltammetry. Both methods have inherent drawbacks, but the generation of a hydrodynamic voltammogram is less complex than the generation of a cyclic voltammogram. In order to obtain data for the construction of a hydrodynamic voltammogram, the potential of the analytical electrode is held constant and the peak height(s) of an analyte(s) are measured. Electrode potentials are gradually increased after allowing the detector to stabilize between increments. The peak height(s) of the compound(s) of interest are then measured at each potential. The voltage is increased to the point where no further increase in peak height(s)

are seen. The peak height(s) are then converted to current and a voltammogram is constructed by plotting current versus applied potential. Operating potentials are then selected at a potential greater than the applied potential where the voltammogram levels off. Hydrodynamic voltammograms generated for cyclizine, protryptiline and chlorcyclizine are depicted in Figure 3.1.

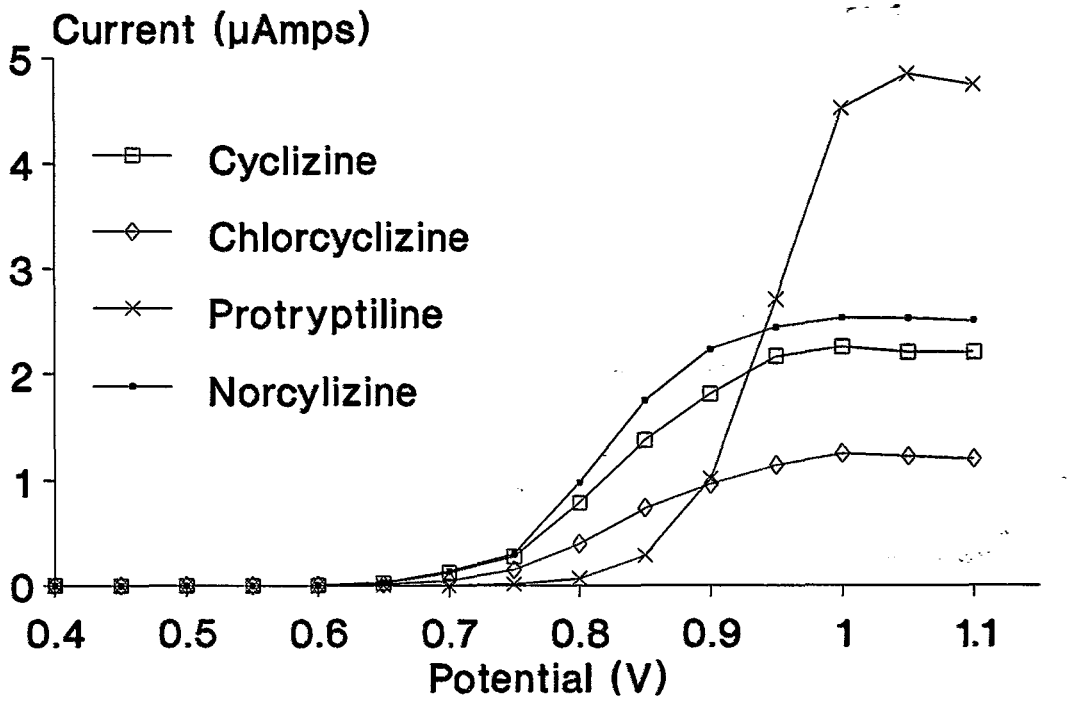


Figure 3.1 Hydrodynamic voltammograms of, cyclizine, protryptiline and chlorcyclizine.

The maximum response obtained for cyclizine when compared with that obtained for an ultraviolet detector, showed a slight increase in sensitivity. However, the use of the dual-electrode coulometric cell can be a more selective method for the determination of these compounds in biological fluids. Goto *et al* (142) have determined the metabolites of several biogenic amines by use of an innovative approach that uses electrochemical detection with two working electrodes operating simultaneously, at different potentials. The advantage of using a dual electrode system is that the first or upstream electrode can be operated at a lower potential, effectively performing a screening function, to oxidize contaminants rendering them undetectable. The second or downstream electrode is set at an optimum potential for the selective detection of the compound(s) of interest. The "oxidative screen-mode" has been used for the analysis of several drugs in biological matrices (139,140,141,143,144,145,146,147). This "oxidative screen-mode" technique was used for the determination of cyclizine and its metabolite, norcyclizine, in serum and urine.

3.1.6 Internal Standard

Protryptiline hydrochloride was selected as the internal standard because of its favourable chromatographic behaviour. Whilst, chlorcyclizine would be a more ideal internal standard, as it has the same basic chemical structure as norcyclizine and cyclizine, it was not used initially due to the prolonged retention time (*ca.* 15 minutes), it exhibited. The lipophilicity of molecules is known to increase as the number of substituted non-polar functional groups (e.g., the halogens) increase (148). The prolonged retention time is likely to be a result of increased lipophilicity of the chlorcyclizine due to the presence of a chlorine atom.

3.1.7 Chromatographic Conditions

1) Mobile-phase flow rate	-1.0ml.min^{-1}
2) Column	-Techsil C_{18} (5μm)
	-Length 150mm
	-I.D. 4.1mm
3) Column temperature	-30°C
4) Column pressure	-10 x 10^6Nm^{-2}
5) Retention times	-Norcyclizine *ca.* 5min.
	-Cyclizine *ca.* 6min.
	-Internal standard *ca.* 8min.
6) Detector settings	-Guard cell potential - 1.00V
	-Analytical cell electrode 1 - 0.55V
	-Analytical cell electrode 2 - 0.90V
7) Injection volume	-5-15$\mu\ell$
8) Chart speed	-2.5mm/min
9) Chart recorder input	-10mV full scale

A typical chromatogram of an aqueous sample of norcyclizine, cyclizine and protryptiline is depicted in Figure 3.2.

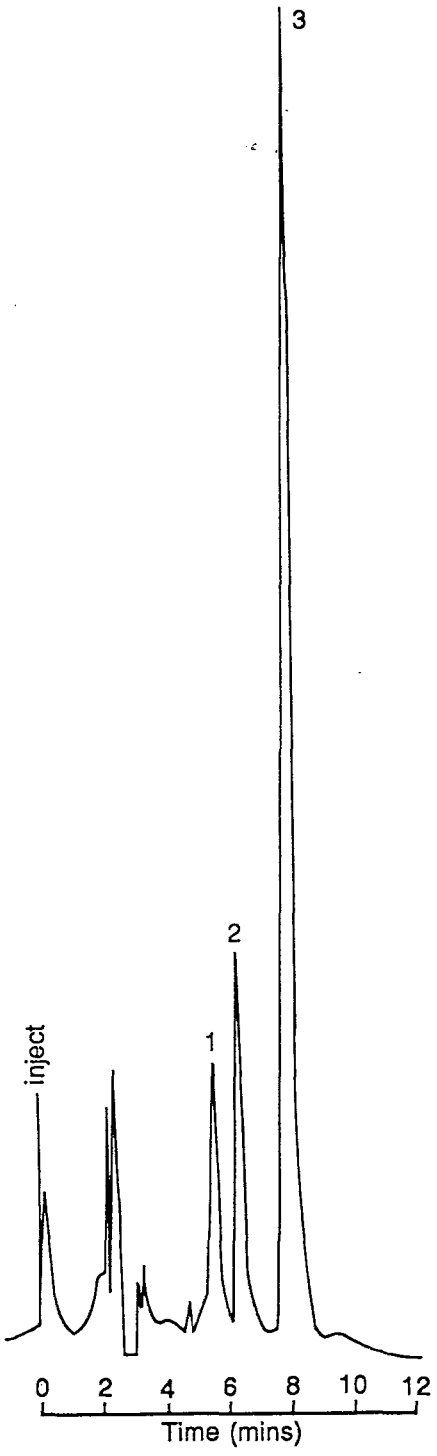


Figure 3.2 Typical chromatogram obtained on a 5μm C_{18} analytical column of an aqueous sample of norcyclizine (1); on column load = 8ng, cyclizine (2); on column load = 6ng, and protryptiline (3); on column load = 26ng. The detector potentials were 0.55V (Det. 1) and 0.90V (Det. 2). Other chromatographic conditions are as specified in § 3.1.7 and mobile-phase as specified in § 3.1.4.

3.2 EXTRACTION OF NORCYCLIZINE AND CYCLIZINE FROM SERUM AND URINE

3.2.1 Method Development

Blank serum was extracted using the method described in § 2.2.2.7. However, samples prepared using this method, showed interfering components at the retention times of norcyclizine and cyclizine. The extraction method was therefore modified by removal of the acetonitrile-0.05M (pH=3) phosphate buffer (70:30) column conditioning wash step. Analysis of blank serum revealed clean extracts with no contaminating peaks. The improvement may have been due to the removal of the conditioning step which provided a suitable environment for the selective retention of electrochemically active compounds that were not evident when analysis was performed using ultraviolet detection.

3.2.3 Validation of the Analytical Method - Serum Extraction

3.2.3.1 Calibration and Linearity

Linearity was established by the preparation and extraction of calibration standards prepared in drug-free serum, spiked with known quantities of cyclizine. In the initial stages of development norcyclizine was not included in the standards. Calibration standards over the concentration range 0-100ng/ml were prepared by dilution of a serum stock solution. An aqueous solution of cyclizine hydrochloride was prepared by accurately weighing 10mg of cyclizine hydrochloride into a 100ml A grade volumetric flask and making up to volume with water. The solution was sonicated for 5 minutes to ensure complete solution of the cyclizine (solution A). A 1ml aliquot of solution A was diluted to 10ml with water yielding a 10μg/ml solution of cyclizine hydrochloride (solution B). The upper standard (100ng/ml) was prepared by pipetting 1ml of solution B into a 100ml volumetric flask and making up to volume with blank serum. The desired standards (70, 50, 20, 10, 5 and 1ng/ml) were prepared by relevant dilutions of the upper standard. Samples were prepared for analysis as described in § 2.2.2.7.

3.2.3.2 Precision and Accuracy

The precision of the assay was determined by calculation of the relative standard deviations of the peak height ratios of the replicate samples of calibration standards, and the accuracy determined by comparison of the theoretical concentration of cyclizine to the concentration determined from the regression equation derived from those standards and reported as the percent bias.

3.2.4 Results and Discussion

3.2.4.1 Calibration and Linearity

A calibration curve was constructed by plotting (Figure 3.3) the peak height ratios of cyclizine to internal standard (protryptiline hydrochloride) versus cyclizine concentration and performing least squares linear regression. The results of the analysis are depicted in Table 3.1. The calibration curve had a slope of 0.008896 and a y-intercept of 0.03496 with a correlation coefficient of 0.9800. The method exhibited a linear response over the concentration range studied.

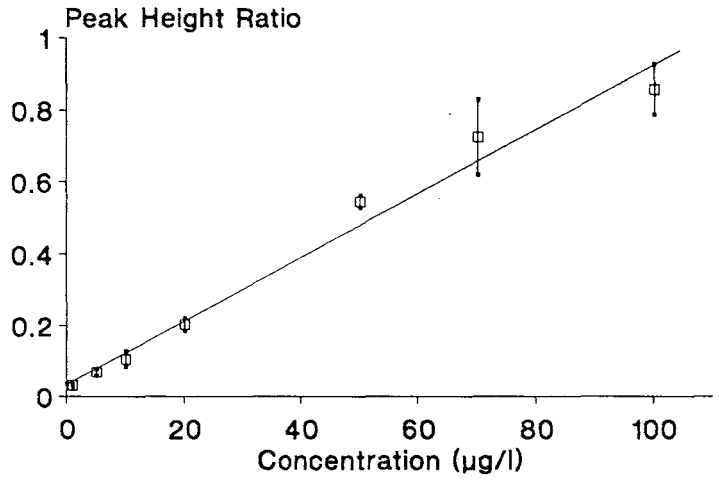


Figure 3.3 Calibration curve constructed after linear regression of mean peak height ratios versus concentration following the extraction of replicate samples of cyclizine in serum. Linear regression equation:- y=0.008896x + 0.03496.

3.2.4.2 Precision and Accuracy

Results from the precision and accuracy study are tabulated in Table 3.1. Accuracy data are recorded as the concentrations found of calibration samples prepared in § 3.2.3.1. It is clearly evident from the results that despite reasonable precision the method is inaccurate and thus not suitable for pharmacokinetic studies.

Table 3.1 Precision and Accuracy Data for the Extraction of Cyclizine from Serum using the Method Described in § 3.2.1.

Calibration and Precision Data				Accuracy Data		
*CYC (ng/ml)	**Base (ng/ml)	***(PHR) (n=6)	R.S.D. %	CYC Found ± S.D. (ng/ml)	Base Found (ng/ml)	% Bias
1.002	0.88	0.031	11.0	0.19 ± 0.17	0.17	- 80.96
5.01	4.41	0.068	14.4	3.73 ± 1.10	3.28	- 25.55
10.02	8.81	0.105	21.0	7.91 ± 2.49	6.96	- 21.06
20.04	17.63	0.202	8.9	18.76 ± 2.02	16.50	- 6.39
50.10	44.08	0.542	3.2	56.94 ± 1.95	50.08	+ 13.65
70.14	61.70	0.724	14.4	77.42 ± 11.74	68.10	+ 10.44
100.20	88.14	0.855	8.1	92.18 ± 7.75	81.08	- 8.00

* Cyclizine hydrochloride concentration
** Cyclizine base equivalent concentration
*** Peak height ratio of cyclizine/internal standard

3.2.5 Discussion

The presence of endogenous amines in biological samples has previously been shown to have posed problems in the analysis of the macrolide antibiotic, josamycin (115) and a 21-aminosteroid anti-oxidant (149). Both authors postulated that the reported partial or complete loss of drug during solid-phase extraction procedures occurred as a result of the presence of endogenous salts and amines. Skinner (115) has demonstrated that the use of a large volume (20ml) water wash after sample loading is both suitable and necessary for the precise and accurate quantitation of josamycin in samples obtained from a volunteer that ingested the compound. The extraction procedure for the determination of cyclizine in serum was therefore modified to include a 20ml water wash after sample loading. The results obtained after re-investigation of linearity, precision and accuracy were in general, better than those reported previously (Table 3.1). The relative standard deviations ranged from 2.46 to 16.00 percent and the percent bias for accuracy from -21.18 to +15.02 percent. These results indicate a more acceptable but not suitable analytical procedure for pharmacokinetic studies. One further advantage of the inclusion of a 20ml water wash after sample loading was the cleanliness of the extract. Investigations into the cause of the imprecision and inaccuracy which occurred when the detection system was changed from UV to electrochemical were therefore undertaken.

3.2.6 Investigations to Determine the Cause of Poor Precision and Inaccuracy

The need for a sensitive and selective analytical method necessitated further investigations into the cause of the poor precision encountered in the analysis of cyclizine in biological fluids. The procedure involved identification and elimination of the possible sources of variability with respect to instrumentation and internal standard selection.

3.2.6.1 HPLC Apparatus

The modular chromatographic system used in these experiments was altered from that previously described (§ 3.1.2). The changes made to the system were implemented to eliminate possible causes of imprecision due to variable injection volumes and possible variation of the detector response over time. The chromatographic system consisted of a Waters Model M 6000A dual piston constant flow pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne Model 7125 sample injector fitted with a 10$\mu\ell$ fixed volume loop (Rheodyne Inc., Cotati, CA, U.S.A), a Model 5100A Coulochem dual electrode electrochemical detector fitted with a model 5010 analytical cell (Environmental Sciences Associates Inc., Bedford, MA, U.S.A.) preceded by a carbon filter. In addition, a Spectraphysics SP 8480XR (Spectra-Physics Inc., San Jose, CA, U.S.A.) variable wavelength ultraviolet detector was placed in line. The data were recorded on a dual-pen Model 561 strip chart recorder (Hitachi Ltd., Tokyo, Japan). The mobile-phase was constantly degassed using an in-line Model ERC-3510 degasser (Erma Optical Works Ltd., Tokyo, Japan). The mobile-phase was "scrubbed" with a Model 5020 guard cell (Environmental Sciences Associates Inc., Bedford, MA, U.S.A.) preceded by a carbon filter. The analytical column was a custom made 15cm x 4.1mm i.d. stainless-steel column, packed with C_{18} (5μm) material (HPLC Technology, Wilmslow, United Kingdom). An Uptight Precolumn Kit (Upchurch Scientific Inc., Oak Harbor, WA, U.S.A.) packed with glass beads was placed in-line before the analytical column. Both the guard and analytical columns were maintained at 30°C with a Model LC-22 temperature controller (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.).

3.2.6.2 Chromatographic Conditions

The chromatographic conditions that were used are documented in § 3.1.7. Specific points of importance are that an injection volume of 10$\mu\ell$ was used throughout the experiment. The use of a fixed volume loop injector eliminates any imprecision that may arise as a result of variable injection volumes. The loop was filled completely by injection of 40$\mu\ell$ of sample. The inclusion of the ultraviolet detector was essential to provide an indication as to whether the detector was the source

of poor precision. The ultraviolet detector was set at a wavelength of 200nm and the sensitivity was set at 0.01 absorbance units full scale (AUFS) for detection of cyclizine (2ng/$\mu\ell$) and chlorcyclizine (3ng/$\mu\ell$) and at 0.04 AUFS for protryptiline (3ng/$\mu\ell$).

3.2.6.3 **Experimental**

3.2.6.3.1 Injector Precision

Injector precision was determined in order to guarantee that the performance of the injector was consistent. Precision was determined by injection of six 10$\mu\ell$ samples of a cyclizine hydrochloride solution and the response monitored at 200nm with the ultraviolet detector. Results of these studies are tabulated in Table 3.2.

3.2.6.3.2 Precision of Detector Response to Cyclizine, Chlorcyclizine and Protryptiline

The precision of detector response was investigated by loading 10$\mu\ell$ of cyclizine, followed by 10$\mu\ell$ protryptiline and finally 10$\mu\ell$ of chlorcyclizine on each of six occasions. In addition, six 10$\mu\ell$ aliquots of cyclizine were injected followed by six aliquots of protryptiline and six aliquots of chlorcyclizine. The samples were monitored using both ultraviolet and electrochemical detection and the results are tabulated in Tables 3.3 and 3.4.

3.2.6.4 **Results and Discussion**

The results precision studies of the Rheodyne injector revealed that by use of a full loop, the analyst can be confident of precise peak height measurements. In both instances the relative standard deviation of the data indicate that variations of up to 1% can be expected if this injection system was used in conjunction with an ultraviolet detector. Therefore use of this injector minimizes any error in precision that might occur due to the method of injection.

Table 3.2 Results of Rheodyne Injector Precision Evaluation using an Ultraviolet Detector.

*Before Detector Precision			*After Detector Precision		
No. of Samples	Mean Peak Height (mm)	R.S.D. %	No. of Samples	Mean Peak Height (mm)	R.S.D. %
6	116	0.59	6	119	1.03

Injector precision determined before and after investigations into detector precision

Any variation in precision that may have occurred during serum sample analysis can thus be attributed to the detector being used. Injection of 10$\mu\ell$ aliquots of each compound in succession revealed that irrespective of the detector used there is close agreement in the percent relative standard deviation for the six injections of cyclizine and chlorcyclizine. However, for protryptiline, there is a large discrepancy between the precision obtained using the ultraviolet detector and that obtained using the electrochemical detector.

Table 3.3 Results Obtained Following Injection of 10$\mu\ell$ Cyclizine, 10$\mu\ell$ Protryptiline and 10$\mu\ell$ Chlorcyclizine in Succession on Each of Six Occasions.

Compound	No. of Samples	*MPH (mm) ECD	**MPH (mm) UV	R.S.D. % ECD	R.S.D. % UV
Cyclizine	6	206	127	2.14	2.51
Protryptiline	6	132	112	15.52	5.22
Chlorcyclizine	6	153	110	7.12	7.83

* Mean peak height of six samples with electrochemical detection.
** Mean peak height of six samples with ultraviolet detection.

Peak height precision following consecutive injections of cyclizine (n=6), protryptiline (n=6) and chlorcyclizine (n=6) was improved for all three compounds. The calculated percent relative standard deviations for cyclizine and chlorcyclizine were in close agreement irrespective of the detector used. However, despite some improvement, there was a large degree of disparity, in the precision, between the two detectors, for protryptiline.

Table 3.4 Results Obtained Following Consecutive Injections of Six 10$\mu\ell$ Samples of Cyclizine, Protryptiline and Chlorcyclizine.

Compound	No. of Samples	*MPH (mm) ECD	**MPH (mm) UV	R.S.D. % ECD	R.S.D. % UV
Cyclizine	6	177	111	0.71	0.67
Protryptiline	6	109	122	9.43	1.90
Chlorcyclizine	6	160	114	2.54	2.05

* Mean peak height of six samples with electrochemical detection.
** Mean peak height of six samples with ultraviolet detection.

The results indicate that the peak height of a series of protryptiline samples varies when using electrochemical detection for analysis. The implications of the variation become obvious if the

compound is to be used as an internal standard. The peak heights of a series of chlorcyclizine samples were far more reproducible. One of the requirements of an internal standard is that it bears a close structural similarity with the drug to be assayed (150). The need for similarity is necessary when chromatographic separations are being developed to ensure similar retention times and extractability are obtained to prevent long analysis times. Most drugs absorb ultraviolet light at low wavelengths (*viz.* 200nm) and therefore a similarity in chemical structure need not be an absolute necessity. These investigations suggest that compounds with similar oxidation characteristics are essential for the development of a precise and accurate analytical method using electrochemical detection.

The electrode potentials of the analytical cell were set at 0.55V for the upstream electrode and 0.90V for the downstream electrode. The hydrodynamic voltammograms (Figure 3.1) for norcyclizine, cyclizine and chlorcyclizine show plateaus at a potential of 0.90V, whereas, that for protryptiline shows a steep inflection. Consequently, any slight variation in the potential would have a marked effect on the recorded peak height for protryptiline. In the interest of greater precision, chlorcyclizine was selected as the internal standard for the analysis of cyclizine by electrochemical detection.

3.3 SERUM EXTRACTION

3.3.1 Validation of the Assay

3.3.1.1 Extraction Procedure

The extraction procedure described (§ 2.2.2.7) was modified in the following manner:-

a) no "elution buffer" conditioning step was used,
b) a 20ml water wash was employed after sample loading, and,
c) chlorcyclizine was used as the internal standard.

Chromatograms of blank and spiked serum samples extracted using this method are depicted in Figure 3.4.

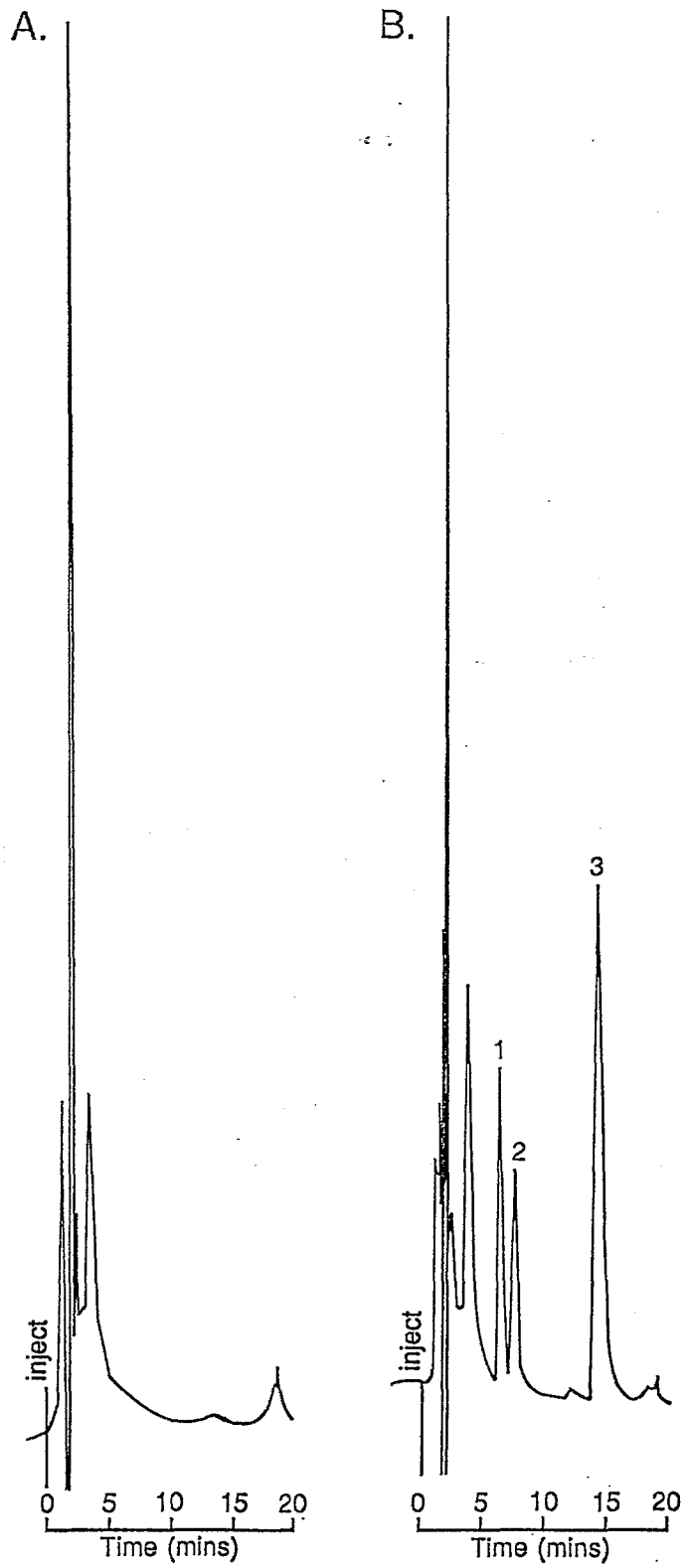


Figure 3.4 HPLC chromatograms of (A) blank human serum and (B) human serum spiked with (1) norcyclizine (20.02ng/ml), (2) cyclizine (20.30ng/ml) and 100ng/ml of (3) internal standard (chlorcyclizine).

3.3.1.2 Calibration and Linearity

Linearity was established by the preparation and extraction of replicate calibration standards (n=6) prepared in drug-free serum, spiked with known quantities of norcyclizine and cyclizine. Calibration standards over the concentration range 0-100ng/ml were prepared as described in § 2.2.5.2 and § 3.2.3.1 and the samples prepared for analysis as described in § 2.2.2.1.

3.3.1.3 Precision and Accuracy

Precision and accuracy were determined as described in § 3.2.3.2.

3.3.1.4 Extraction Efficiency

The extraction efficiency was determined using the procedure described in § 2.2.3.3 at concentrations of 5.0, 50.0 and 100.0ng/ml.

3.3.2 Results and Discussion

3.3.2.1 Calibration and Linearity

Calibration curves were constructed by linear regression of plots of peak height ratios of either norcyclizine or cyclizine and internal standard versus concentration of norcyclizine (Figure 3.5) or cyclizine (Figure 3.6) and were found to be linear over the calibration range (0-100ng/ml) studied The results of the analysis are depicted in Tables 3.5 and 3.6.

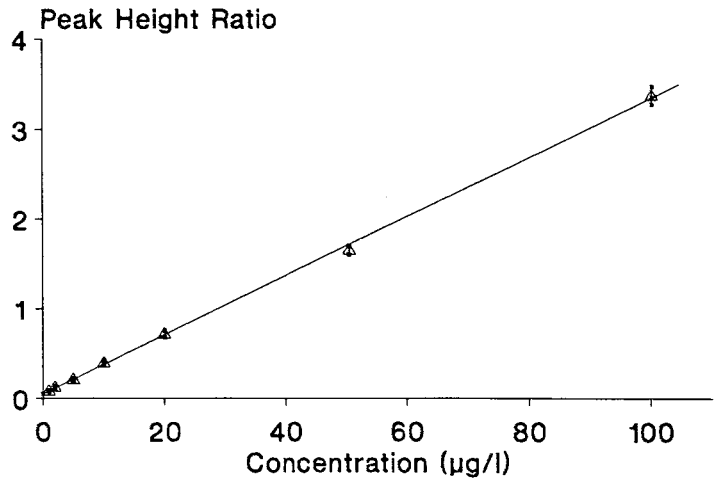


Figure 3.5 Calibration curve constructed for norcyclizine after linear regression of mean peak height ratios versus concentration after extraction of replicate samples. Linear regression equation:- y=0.0329x + 0.0501.

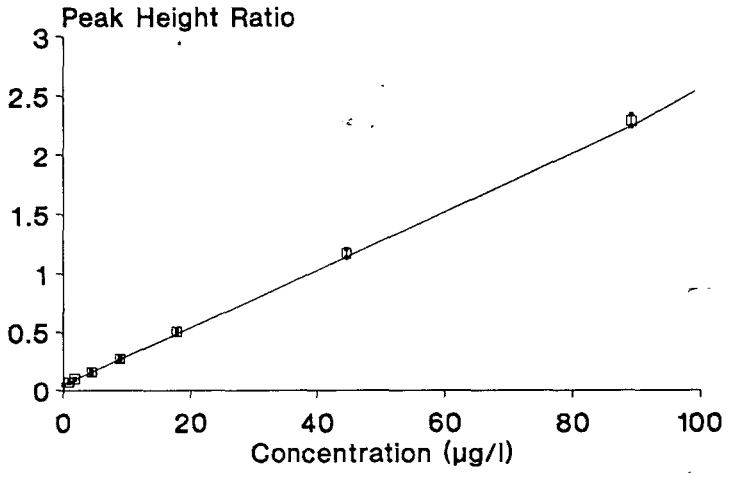


Figure 3.6 Calibration curve constructed for cyclizine after linear regression of mean peak height ratios versus concentration after extraction of replicate samples. Linear regression equation:- $y=0.0251x + 0.0505$.

3.3.2.2 Precision and Accuracy

Results from the precision and accuracy studies are tabulated in Tables 3.5 and 3.6. In all cases RSD values were less than 10 percent for both norcyclizine and cyclizine. The accuracy data are reported as concentration found of known standards. In all but one case for norcyclizine and in all cases for cyclizine the percent bias or accuracy was $\leq$ 10%. These data conform with the criteria laid down by Peng and Chiou (123) indicating that the assay is suitable for pharmacokinetic studies.

Table 3.5 Precision and Accuracy Data for the Extraction of Norcyclizine from Serum using the Method Described in § 3.3.1.1.

Calibration and Precision Data			Accuracy Data	
*NCYC (ng/ml)	**(PHR) (n=6)	R.S.D. %	NCYC Found ± S.D. (ng/ml)	% Bias
1.001	0.0835	5.58	1.02 ± 0.14	+ 1.89
2.002	0.126	9.53	2.31 ± 0.37	+ 15.38
5.005	0.211	6.29	4.89 ± 0.40	- 2.30
10.10	0.398	4.71	10.56 ± 0.57	+ 5.49
20.02	0.715	4.80	20.18 ± 1.04	+ 0.80
50.05	1.644	2.89	48.39 ± 1.44	- 4.18
100.10	3.371	2.93	100.84 ± 3.03	+ 0.74

* Norcyclizine concentration
** Peak height ration of norcyclizine/internal standard

Table 3.6 Precision and Accuracy Data for the Extraction of Cyclizine from Serum using the Method Described in § 3.3.1.1.

Calibration and Precision Data				Accuracy Data		
*CYC (ng/ml)	**Base (ng/ml)	***(PHR) (n=5)	R.S.D. %	CYC Found ± S.D. (ng/ml)	Base Found (ng/ml)	% Bias
1.015	0.893	0.0716	6.78	0.959 ± 0.18	0.844	- 5.47
2.03	1.79	0.0993	3.46	2.21 ± 0.16	1.94	+ 8.62
5.075	4.46	0.156	4.96	4.80 ± 0.35	4.22	- 5.47
10.15	8.93	0.270	4.34	9.96 ± 0.53	8.76	- 1.88
20.30	17.86	0.507	3.33	20.68 ± 0.76	18.19	+ 1.85
50.75	44.64	1.173	3.15	50.89 ± 1.67	44.76	+ 0.27
101.50	89.28	2.288	2.31	101.36 ± 2.40	89.16	- 0.13

* Cyclizine hydrochloride concentration
** Cyclizine base equivalent concentration
*** Peak height ratio of cyclizine/internal standard

3.3.2.3 Extraction Efficiency

Results of extraction efficiency studies are tabulated in Table 3.7. The mean recovery values for norcyclizine and cyclizine from serum were 65% and 67% respectively. Whilst a high recovery is desired for sensitivity, consistent recovery will ensure precision and accuracy of the method. The higher losses experienced using this extraction procedure can probably be accounted for by the change in wash sequence and failure of the compounds to partition during the phase-separation step.

3.3.2.4 Limit of Quantitation (LOQ)

The limit of quantitation based on a relative standard deviation and percentage bias of $\leq$ 10% was found to be 1ng/ml. This corresponds to an on-column load of 0.5ng of either norcyclizine or cyclizine in a 15$\mu\ell$ injection using 50% of the final reconstitution. The electrochemical detector analytical cell electrodes were set at 0.55V (detector 1) and 0.90V (detector 2) with a gain of 80.

Table 3.7 Extraction Efficiency Data after the Extraction of both Norcyclizine and Cyclizine from Serum using the Method Described in § 3.3.1.1.

Spiked Concentration (ng/ml)	Aqueous Reference Samples (n=5) *PHR ± R.S.D. %	Extracted Serum Samples (n=5) *PHR ± R.S.D. %	Percentage Recovery
Norcyclizine			
5	0.0553 ± 2.37	0.0393 ± 6.24	71
50	0.522 ± 4.28	0.309 ± 6.33	59
100	0.989 ± 7.41	0.628 ± 9.12	64
Mean ± S.D.			65 ± 5
Cyclizine			
5	0.0537 ± 2.67	0.0362 ± 5.10	68
50	0.531 ± 1.51	0.310 ± 0.87	74
100	1.057 ± 1.72	0.637 ± 3.72	60
Mean ± S.D.			67 ± 6

*Peak height ratio of norcyclizine or cyclizine/internal standard

3.4 URINE EXTRACTION

3.4.1 Validation of the Assay

3.4.1.1 Extraction Procedure

Urine samples were extracted using the method validated for the extraction of norcyclizine and cyclizine from serum (§ 3.3.1.1). Urine samples were monitored for both cyclizine and its inactive demethylated metabolite, norcyclizine. Clean extracts were obtained without further modification of the extraction procedure and chromatograms of blank and spiked urine samples prepared using the method described are depicted in Figure 3.7.

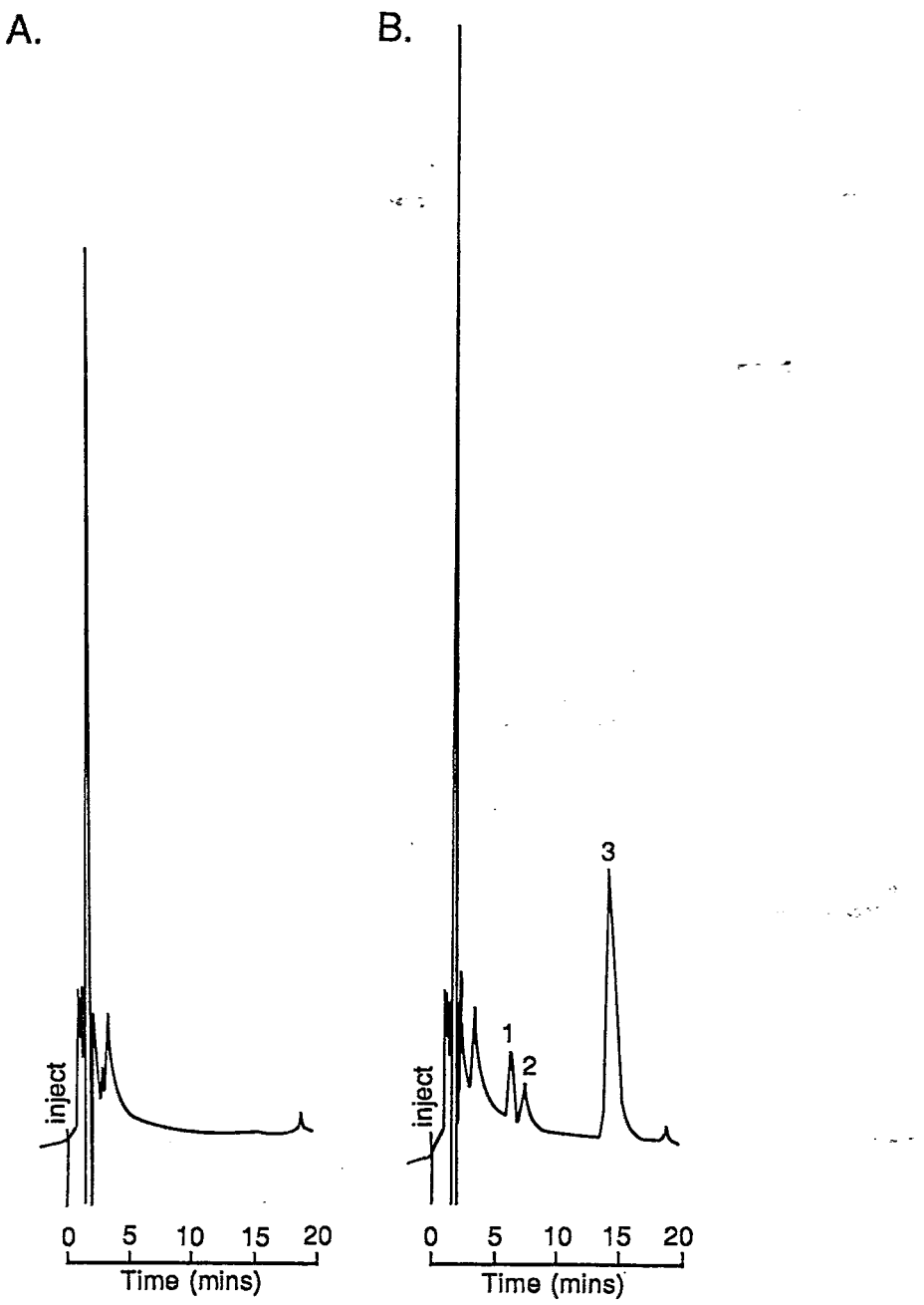


Figure 3.7 HPLC chromatograms of (A) blank human urine and (B) human urine spiked with (1) norcyclizine (8.01ng/ml), (2) cyclizine (8.22ng/ml) and 200ng/ml of (3) internal standard (chlorcyclizine).

3.4.1.2 Calibration and Linearity

Linearity was established by the preparation and extraction of calibration standards that had been prepared using drug-free urine with known quantities of both cyclizine and norcyclizine. Previously (§ 2.2.5.2) calibration standards over the range 0-100ng/ml were prepared. However, results of an 8 hour urine study (§ 2.4.3.2) indicated that concentrations of both cyclizine and norcyclizine were likely to exceed 100ng/ml. Therefore, calibration standards over the concentration range 0-400ng/ml were prepared by dilution of a urine stock solution containing norcyclizine and cyclizine. Aqueous stock solutions of cyclizine hydrochloride and norcyclizine were prepared as previously described

in § 2.2.5.2. The upper standard was prepared by pipetting 2ml of each of the norcyclizine and cyclizine solutions and diluting to 50ml with blank urine. The desired standards (200, 120, 80, 20 and 8ng/ml) were prepared by appropriate dilution of the upper standard. A 500$\mu\ell$ aliquot of each solution was extracted.

3.4.1.3 Precision and Accuracy

Precision and accuracy were assessed as described in § 3.2.3.2.

3.4.1.3 Extraction Efficiency

The percentage recovery from spiked urine samples was determined as detailed in § 2.2.3.3. Replicate samples of urine solutions containing 40ng/ml and 400ng/ml of both cyclizine and norcyclizine were extracted.

3.4.2 Results and Discussion

3.4.2.1 Calibration and Linearity

Calibration curves were constructed by plotting peak height ratios of norcyclizine (Figure 3.8) and cyclizine (Figure 3.9) to internal standard versus concentration and performing least squares linear regression. The results of this analysis are tabulated in Tables 3.8 and 3.9. The calibration curves had slopes of 0.00538 and 0.00414, y-intercepts of -0.00194 and 0.00251 and correlation coefficients of 0.9993 and 0.9998 for norcyclizine and cyclizine respectively.

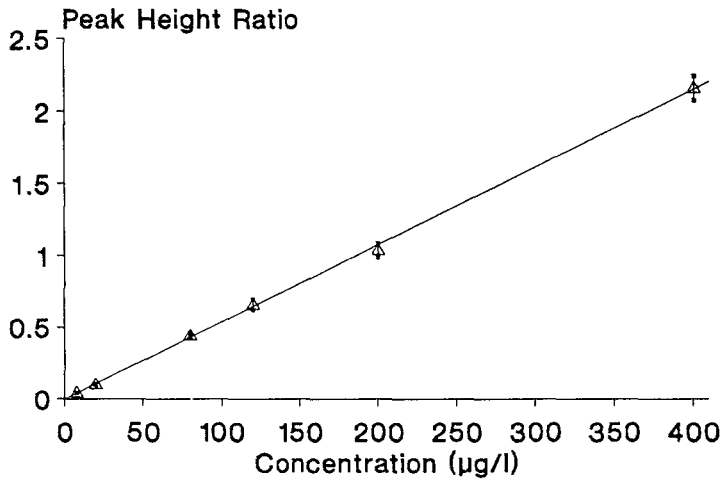


Figure 3.8 Calibration curve constructed after linear regression of mean peak height ratios versus concentration after the extraction of replicate samples of norcyclizine in urine. Linear regression equation:- y=0.00538x - 0.00194.

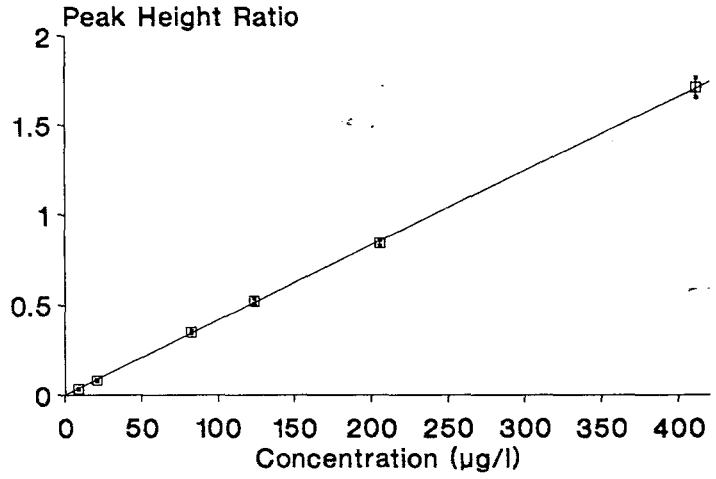


Figure 3.9 Calibration curve constructed after linear regression of mean peak height ratios versus concentration after the extraction of replicate samples of cyclizine in urine. Linear regression equation:- $y=0.00414x + 0.0251$.

3.4.2.2 Precision and Accuracy

Table 3.8 lists the results of precision and accuracy studies for the determination of cyclizine and Table 3.9 the results for norcyclizine in urine. Accuracy data are recorded as concentration found of known standards and in all cases the percent bias and precision were $\leq$ 10%. The assay is therefore suitable for the accurate and precise determination of cyclizine and norcyclizine in urine and appropriate for pharmacokinetic studies.

Table 3.8 Precision and Accuracy Data for the Extraction of Cyclizine from Urine using the Method Described in § 3.4.1.1.

Calibration and Precision				Accuracy		
*CYC (ng/ml)	**Base (ng/ml)	***PHR (n=3)	R.S.D. %	CYC Found ± S.D. (ng/ml)	Base Found (ng/ml)	% Bias
8.22	7.23	0.0432	0.82	8.04 ± 0.07	7.07	-2.23
20.56	18.08	0.0831	3.2	19.46 ± 0.64	17.12	-5.33
82.24	72.34	0.352	1.3	84.44 ± 1.12	74.27	+2.67
123.36	108.51	0.520	3.4	124.91 ± 4.27	109.87	+1.25
205.60	180.85	0.842	2.1	202.55 ± 4.25	178.16	-1.49
411.20	361.69	1.709	3.3	411.98 ± 13.40	362.38	+0.19

* Cyclizine hydrochloride concentration
** Cyclizine base equivalent concentration
*** Peak height ratio of cyclizine/internal standard

Table 3.9 Precision and Accuracy Data for the Extraction of Norcyclizine from Urine using the Method Described in § 3.4.1.1.

Calibration and Precision			Accuracy	
♦NCYC (ng/ml)	♦♦PHR (n=3)	R.S.D. %	NCYC Found ± S.D. (ng/ml)	% Bias
8.008	0.0432	1.9	8.39 ± 0.15	+4.78
20.02	0.100	4.2	19.00 ± 0.78	-5.08
80.08	0.439	3.8	82.10 ± 2.92	-2.52
120.12	0.657	5.7	122.63 ± 6.92	+2.09
200.20	1.038	4.8	193.25 ± 9.31	-3.47
400.40	2.163	3.9	402.42 ± 15.66	+0.50

♦ Norcyclizine concentration
♦♦ Peak height ratio of norcyclizine/internal standard

3.4.2.3 Extraction Efficiency

The results from percentage recovery studies are tabulated in Table 3.10. Mean recovery values for cyclizine and norcyclizine from urine were 70% and 67% respectively. The loss of both compounds may be a result of the increased volume of the water wash after loading and due to incomplete partitioning of the compounds from the water into the acetonitrile at the phase-separation step.

Table 3.10 Extraction Efficiency Data after the Extraction of both Cyclizine and Norcyclizine from Urine using the Method Described in § 3.4.1.1.

Spiked Sample Concentration (ng/ml)	Aqueous Reference Samples (n=5) *PHR ± R.S.D. %	Extracted Urine Samples (n=5) *PHR ± R.S.D. %	Percentage Recovery
Cyclizine			
40	0.233 ± 4.35	0.157 ± 5.66	67
400	2.010 ± 0.81	1.443 ± 1.89	72
Mean ± S.D.			70 ± 3
Norcyclizine			
40	0.323 ± 2.94	0.241 ± 5.24	73
400	3.125 ± 0.50	1.888 ± 2.39	60
Mean ± S.D.			67 ± 7

* Peak height ratio of cyclizine or norcyclizine/internal standard

3.4.3 Discussion

A rapid and sensitive assay with the precision and accuracy necessary for pharmacokinetic studies has been developed for the analysis of both cyclizine and its demethylated metabolite, norcyclizine, in both serum and urine. Previously reported studies were unable to isolate norcyclizine from these biological matrices with any confidence. The use of a dual electrode electrochemical detector operated in the "oxidative-screen" mode allowed the selective quantitation of norcyclizine and cyclizine from both serum and urine. SPE provided a suitable means to extract between 80 and 100 samples within 6-8 hours despite the large water wash that was necessary to improve both the cleanliness and precision of the extraction process. One important aspect of electrochemical detector use in the analysis of cyclizine, is that the choice of internal standard appears to be critical. Despite similarities in retention properties, the internal standard must have similar oxidation characteristics.

Whilst both methods reported in this dissertation have the necessary characteristics of precision and accuracy for use in pharmacokinetic studies of cyclizine, the need to quantitate low levels of the drug following therapeutic doses required a highly sensitive and selective analytical method. The use of ultraviolet detection necessitates monitoring the eluate at a wavelength of 200nm, a region in the ultraviolet spectrum known to be no- discriminating. The use of an electrochemical detector, more specifically a coulometric detector with a dual electrode analytical cell, allowed for selective quantitation of both cyclizine and norcyclizine in the biological fluids collected. Selectivity was enhanced by operation of the cell in the "oxidative-screen" mode, in which the upstream electrode effectively acts as a filter, oxidizing compounds that may interfere with the chromatography of the compounds of interest. In addition, the LOQ of both norcyclizine and cyclizine was $1\mu g/\ell$ as opposed to the $5\mu g/\ell$ using ultraviolet detection.

CHAPTER 4

DISSOLUTION AND DOSAGE FORM ANALYSIS

Pharmaceutical manufacturers endeavour to produce safe and effective pharmaceutical dosage forms for use by the general population. In order to achieve these objectives, quality control tests on the products in question must be performed. Whilst *in vitro* or laboratory tests will not guarantee safety and efficacy in the biological system, they do afford the manufacturer the opportunity of pre-empting possible problems with respect to drug use.

Compressed tablets are the most widely used dosage form for ease, accuracy and convenience of manufacture, handling and administration. A pre-requisite for gastro-intestinal absorption of drugs is that drug molecules must be in solution. Therefore, a solid dosage form must liberate the drug which should dissolve in order to facilitate absorption before a desired pharmacological effect will ensue. Drug absorption from orally administered solid dosage forms can be affected by complex designs of tablet formulations and the intrinsic solubility of the compound. These factors may hinder the onset of pharmacological action, and therefore dosage forms must of necessity be tested to eliminate possible aberrations in *vivo* activity.

Content uniformity assessment confirms accuracy of manufacturing processes and consequently ensures that accurate doses are administered to patients from individual unit dosage forms. Content uniformity determinations are essential as quality control tests and an assessment of the content uniformity of Valoid tablets containing 50mg cyclizine hydrochloride (Wellcome (Pty) Limited, Johannesburg, Republic of South Africa) was performed prior to their use in bioavailability studies.

Compendial requirements for dissolution testing provide minimum requirements for solid dosage forms (151), however, no uniform method exists to test all solid dosage forms. In addition, dissolution cannot be considered a predictor of therapeutic efficiency, despite reported success of several *in vitro-in vivo* correlation studies (152). Dissolution testing is currently considered the single most useful *in vitro* method for assuring batch-to-batch uniformity (153). A dissolution study on Valoid tablets was undertaken prior to bioavailability and pharmacokinetic studies to determine the likelihood of formulation factors affecting the absorption of cyclizine after oral dosing of the tablets.

One aspect of dissolution testing that poses a problem is the variety of apparatuses that have been used. Pernarowski (154) noted that over 150 different apparatus designs each with associated

advantages and disadvantages have been used. Other authors (155,156,157) have comprehensively described these apparatuses in review articles. Previously, official compendia, in the United States (4) and the United Kingdom (3), described two official methods - the rotating basket method and the paddle method. Recently, several different apparatuses for dissolution testing have been included in the British Pharmacopoeia (B.P.) (158) and in the United States Pharmacopoeia (USP) (159). The former compendium makes provision only for dissolution testing of tablets and capsules specifying and describing the basket apparatus (Apparatus 1), the paddle apparatus (Apparatus 2) and the flow-through cell (Apparatus 3), whilst the USP includes the above and in addition specifies and describes apparatuses for extended-release products (Apparatus 3) and a flow-through cell identical to the B.P. (Apparatus 4). Currently, several monographs of pharmaceutical dosage forms listed in these compendia alleviate the problem of apparatus selection by specification of a particular method for dissolution testing of that product.

4.1 DISSOLUTION TESTING OF VALOID TABLETS

The ideal dissolution test must provide a simple assessment for the detection of possible changes in formulation of a drug product and thereby ensure batch-to-batch uniformity (160). The dissolution test indicates that a specified portion of drug dissolves within a designated period of time in a given volume of dissolution medium, in which the dosage form is immersed, and agitated under controlled conditions. Disintegration and dissolution related effects on bioavailability of Valoid tablets were not expected and therefore comprehensive dissolution studies over the gastro-intestinal pH range were not performed.

The analysis of dissolution samples by HPLC may be preferable to an ultraviolet spectrophotometric technique if excipient interference is expected or where stability of drug or dosage form may be a consideration (160). In addition, HPLC allows for the accurate analysis of dissolution samples obtained from pharmaceutical compounds with low doses of active components.

4.1.1 Dissolution

4.1.1.1 Dissolution Apparatus and Medium

The U.S.P. (4) monograph for tablets containing cyclizine hydrochloride specifies that dissolution studies of the dosage form be undertaken with apparatus 2, in 900ml of water agitated at 50 rpm. Apparatus 2, allows for agitation of the dissolution medium with a paddle stirring element. The water bath of the dissolution apparatus (Pharmatest Type PTWS II, Hainburg, Germany) was allowed to

equilibrate to 37°C prior to commencement of the dissolution test. Preheated de-gassed dissolution medium was placed into each flask, and allowed to equilibrate to 37°C whilst being stirred at 50rpm. The flasks were sealed to prevent evaporation of the dissolution fluid. Prior to the introduction of the test sample, bubbles that had formed in the flask were removed by rapid stirring of the dissolution fluid.

4.1.1.2 Dissolution Testing of Test Tablets

The U.S.P. (4) monograph for cyclizine hydrochloride tablets stipulates that no less than 75% of the labelled amount should dissolve in 45 minutes. Samples (50$\mu\ell$) of the dissolution fluid were removed immediately before the tablet was placed in the dissolution vessel and then every minute for 10 minutes and finally at 5 minute intervals for 45 minutes. Samples were removed with the aid of a 100$\mu\ell$ variable transferpettor (Rudolph Brand GMBH and Co., Wertheim-Glashütte, Germany). Samples were removed by insertion of the transferpettor to its maximum depth via a sampling port in the dissolution flask cover thereby ensuring that samples were removed from the same point within the dissolution medium on each occasion. Less than 1ml of dissolution medium was removed from each vessel and dissolution fluid was not replaced during each run.

4.1.2 Sample Preparation and Analysis for Dissolution Studies

4.1.2.1 Preparation of Standards

Calibration standards were prepared such that sample concentrations spanned the concentration that would be found if total dissolution of cyclizine hydrochloride from Valoid tablets was to occur. An aqueous stock solution of cyclizine hydrochloride (0.12mg/ml) was prepared by accurately weighing 12mg of cyclizine hydrochloride into a 100ml volumetric flask and making up to volume with distilled water (solution A). Solution A was sonicated for 5 minutes to ensure that complete solution of the cyclizine had occurred. Calibration standards were prepared by pipetting 1, 2, 5 and 6 millilitre aliquots of solution A into a 10ml volumetric flask and a 1ml aliquot of solution A into a 20ml volumetric flask. A 50$\mu\ell$ aliquot of each standard was added to 50$\mu\ell$ of internal standard solution (§ 4.1.2.2) in an Eppendorf micro-test tube (Eppendorf-Netheler-Hinz GMBH, Hamburg, Germany) and vortexed for 30 seconds. After mixing the solutions were transferred to a WISP limited volume insert (Waters Assoc., Milford, MA, U.S.A.) and replicate 2$\mu\ell$ samples analyzed by HPLC.

4.1.2.2 **Internal Standard Solution**

An internal standard solution of chlorcyclizine hydrochloride was prepared by accurately weighing 60mg of drug and making up to volume in a 1000ml volumetric flask. The solution was placed in an ultrasonic bath for 5 minutes to ensure complete dissolution of chlorcyclizine hydrochloride had occurred.

4.1.2.3 **Preparation of Samples**

Samples (50$\mu\ell$) were removed at the specified times and added to 50$\mu\ell$ of internal standard solution (§ 4.1.2.2) that had been pipetted into a 1.5ml Eppendorf micro-test tube (Eppendorf-Netheler-Hinz GMBH, Hamburg, Germany) and mixed by vortexing for 30 seconds. Insoluble particles in the sample were removed by centrifuging at 12000 rpm for 1 minute in an Eppendorf Model 5414 high speed centrifuge (Eppendorf-Netheler-Hinz GMBH, Hamburg, Germany). An aliquot of the supernatant was transferred to a WISP limited volume insert (Waters Assoc., Milford, MA, U.S.A.) and 2$\mu\ell$ injected onto the chromatograph.

4.1.2.3 **Sample Analysis**

Samples obtained from dissolution studies of each of six Valoid tablets were analyzed by HPLC (§ 3.1.7). The electrochemical detector was used in the "oxidative screen" mode with the upstream electrode set at 0.50V and analytical electrode set at 0.90V with a gain of 40.

4.1.3 <u>Results and Discussion</u>

4.1.3.1 **Calibration and Linearity for Dissolution Studies**

Proof of linearity of the chromatographic system was obtained by plotting peak height ratios of cyclizine hydrochloride to internal standard versus equivalent amount of cyclizine hydrochloride in 900ml of dissolution medium and least squares linear regression analysis performed. The results of this analysis are depicted in Table 4.1. The calibration curve had a slope of 0.0429 and a y-intercept of 0.0727 with a correlation coefficient of 0.9990.

4.1.3.2 **Precision and Accuracy for Dissolution Studies**

Precision and accuracy results are tabulated in Table 4.1. In all cases the precision, indicated by relative standard deviation were ≤ 5% and percent bias ≤ 10%, indicating that the analytical method is both precise and accurate and suitable for use in dissolution studies of cyclizine hydrochloride tablets.

Table 4.1 Precision and Accuracy Data for Dissolution Studies of Valoid Tablets.

Calibration and Precision			Accuracy	
*CYC (mg)	**PHR (n=10)	R.S.D. %	CYC Found ± S.D. (mg)	% Bias
5.4	0.326	3.6	5.90 ± 0.27	+ 9.26
10.8	0.484	3.5	9.85 ± 0.39	- 8.80
21.6	1.029	2.6	22.28 ± 0.63	+ 3.15
54.0	2.416	3.3	54.60 ± 1.86	+ 1.11
64.8	2.829	1.6	64.23 ± 1.04	- 0.879

* Equivalent amount of cyclizine hydrochloride in 900ml of water
** Peak height ratio of cyclizine hydrochloride/internal standard

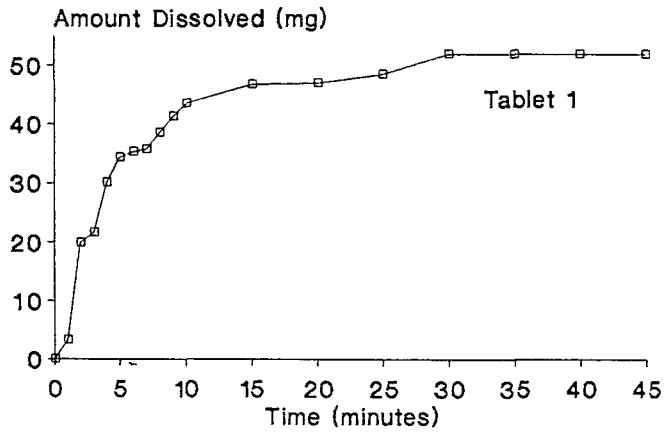
4.1.3.3 **Dissolution Profiles of Valoid Tablets**

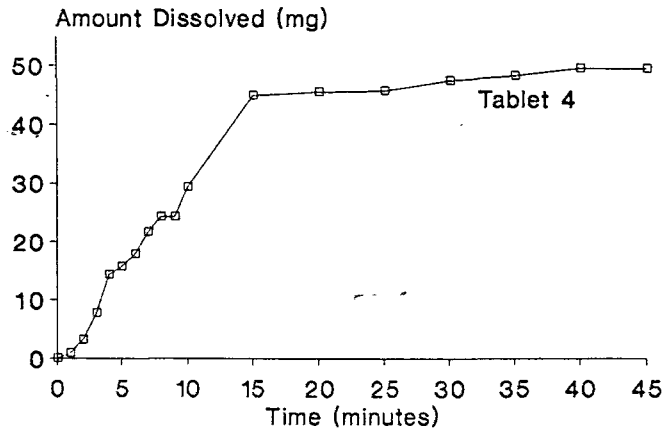
Individual and mean dissolution profiles for Valoid tablets from 0 to 45 minutes are depicted in Figures 4.1 and 4.2 respectively. The dissolution of cyclizine hydrochloride from Valoid tablets is both rapid and complete and all tablets tested fulfilled the U.S.P. requirements that 75 percent of the labelled content be dissolved in 45 minutes. The time taken to reach 50 percent dissolution (fd=0.5 where fd is the fraction dissolved) and the maximum amount released for individual tablets are depicted in Table 4.2.

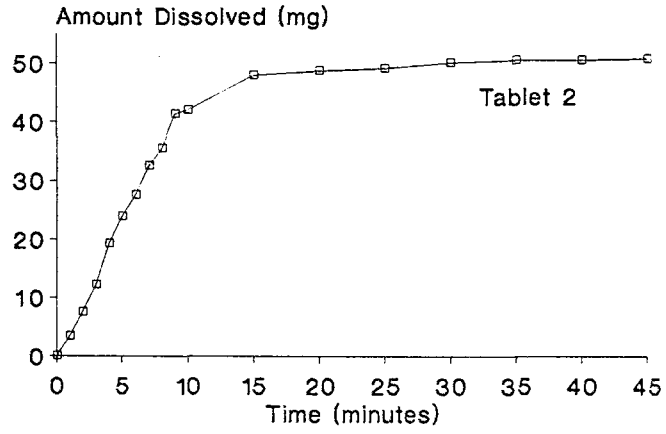
Table 4.2 Dissolution Data for Valoid Tablets Containing 50mg Cyclizine Hydrochloride.

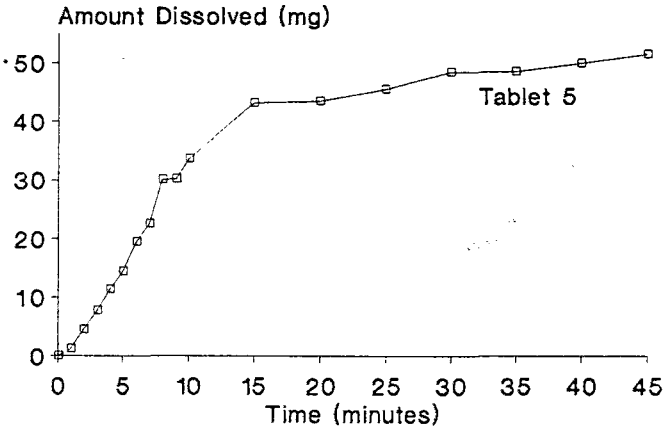
Parameter	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 5	Tablet 6
*fd=0.5 (min)	3.5	5.5	5.5	8.5	7.5	6.5
Maximum Amt. Released at 45 min (mg)	51.95	50.81	52.54	49.55	51.55	48.11

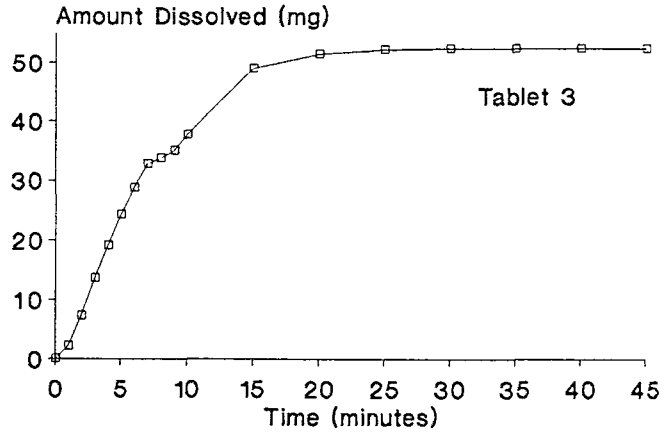
* Time at which the fraction of cyclizine hydrochloride dissolved is 50%.











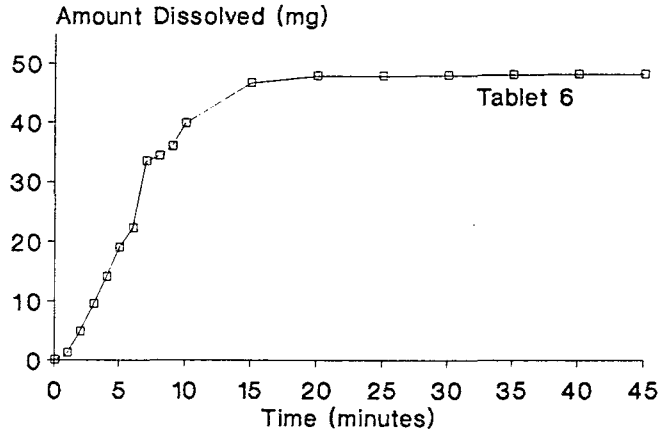


Figure 4.1 Dissolution profiles of individual Valoid tablets. Dissolution tests were performed using U.S.P. apparatus 2, in a dissolution medium of 900ml water, at 50 rpm.

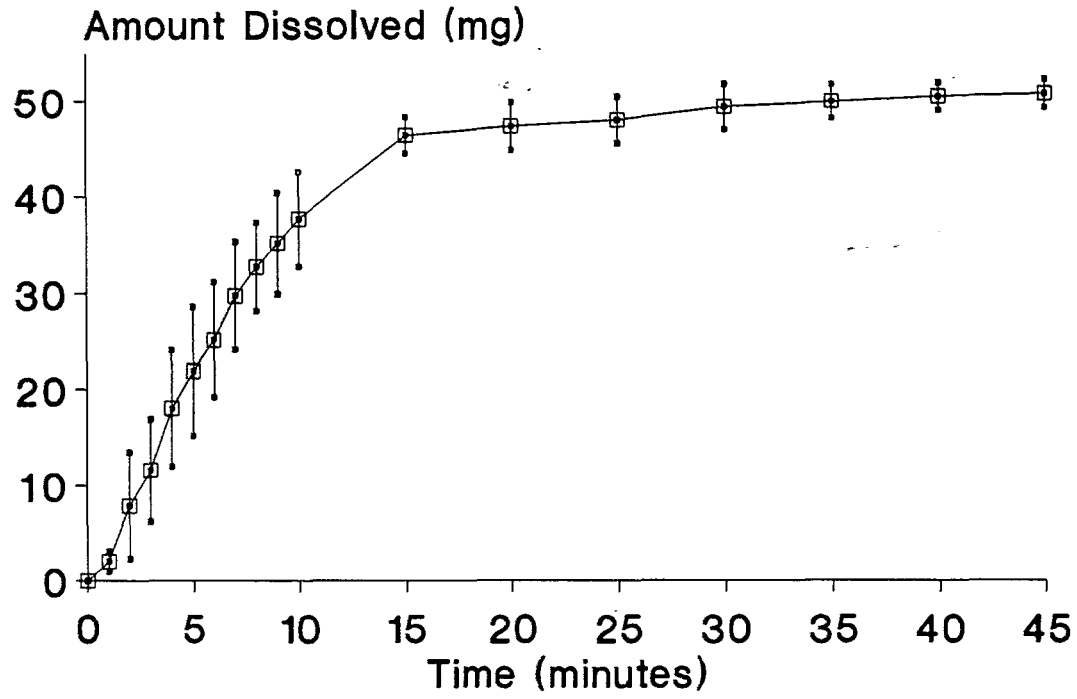


Figure 4.2 Mean dissolution profile of Valoid tablets (N=6). Dissolution tests were performed with U.S.P apparatus 2, in a dissolution medium of 900ml water, at 50 rpm.

4.2 DOSAGE FORM ANALYSIS

Dosage form analysis is an essential requirement for the appraisal of product quality. The determination of content and subsequent uniformity of content within a batch, and between batches of tablets, of the same drug ensures that patients receive both safe and effective products. Official compendia in the United Kingdom and the United States of America specify a range of acceptable content, in terms of percent labelled amount, that is deemed suitable for cyclizine hydrochloride tablets and cyclizine lactate injection. The U.S.P. (4) range for cyclizine hydrochloride tablets is 93 to 107 percent and for cyclizine lactate injection 95 to 105 percent.

Two dosage forms (*viz.* Valoid tablets and Valoid injection) were to be tested in order to elucidate pharmacokinetic parameters and assess the oral bioavailability of cyclizine. Valoid tablets contain 50mg cyclizine hydrochloride and the injection 50mg as cyclizine lactate. The tablets and injection were analyzed by HPLC (§ 3.1.7) with electrochemical detection.

4.2.1 Experimental

4.2.1.1 Preparation of Standards

An aqueous solution of cyclizine hydrochloride was prepared by accurately weighing 10mg of the drug into a 100ml volumetric flask and making up to volume with water (solution A). Solution A was placed in an ultrasonic bath for 5 minutes to ensure that complete dissolution of the cyclizine hydrochloride had occurred. A stock solution of cyclizine hydrochloride was prepared by pipetting a 5ml aliquot of solution A into a 50ml volumetric flask and making to volume with water (solution B). Calibration standards (9, 7, 5, 2, 1ng/$\mu\ell$) were prepared by appropriate dilution of solution B. Internal standard solution was added to each calibration standard as described in § 4.2.1.2. Replicate injections of 2$\mu\ell$ of each standard were injected onto the chromatographic system.

4.2.1.2 Internal Standard Solution

A stock solution (100ng/$\mu\ell$) of chlorcyclizine hydrochloride was prepared by accurately weighing 20mg of drug into a 200ml volumetric flask and making up to volume with water (solution A). To ensure complete dissolution of the chlorcyclizine had occurred, the solution was sonicated for 5 minutes. A 1ml aliquot of solution A was added to each of the calibration standard solutions before each standard was made up to volume. A 10ml aliquot of solution A was added to each of six 100ml volumetric flasks that contained 1ml of sample drug solution, that had been prepared as described in § 4.2.1.3, and made up to volume with water.

4.2.1.3 Sample Preparation (Tablets and Ampoules)

Each of six Valoid tablets were placed into a 100ml volumetric flask and allowed to disintegrate and dissolve in 50ml of water, for 10 minutes, with the aid of an ultrasonic bath. The samples were then made up to volume with water and sonicated for a further 5 minutes. An aliquot of each solution was placed into a 1.5ml micro-test tube and centrifuged at 12000 rpm for 5 minutes to remove insoluble tablet excipients from the solution. A 1ml aliquot of each solution was pipetted into each of six 100ml volumetric flasks containing 10ml of internal standard solution A and made up to volume with water. A 100$\mu\ell$ aliquot of each solution was transferred to each of 3 WISP limited volume inserts (Waters Assoc., Milford, MA, U.S.A.) and a 2$\mu\ell$ sample of each injected onto the chromatographic system. The theoretical on-column load of cyclizine hydrochloride and chlorcyclizine hydrochloride from each sample was 10 and 20ng respectively.

A similar procedure was followed for the analysis of Valoid ampoules. However, as these ampoules contain cyclizine lactate solution the need for sonication and centrifugation to promote disintegration and separation of insoluble excipients, as for tablets, is unnecessary. The contents of six 1ml ampoules were added to each of six 100ml volumetric flasks. The neck and body of each of the ampoules were rinsed several times and the washings added to the volumetric flask containing the contents of the corresponding ampoule. The solutions were made up to volume with water and the contents mixed thoroughly. Preparation and analysis of the cyclizine lactate solutions were completed as for the tablet samples.

4.2.1.4 Sample Analysis

Calibration standards, tablet and ampoule samples were analyzed by HPLC with electrochemical detection (§ 3.1.7). The detector electrodes were operated as described in § 4.1.2.3.

4.2.2 Results and Discussion

4.2.2.1 Calibration and Linearity for Dosage Form Analysis

Calibration curves were constructed by plotting peak height ratio of cyclizine hydrochloride to internal standard versus concentration (for tablets) and peak height ratio of cyclizine hydrochloride to internal standard versus equivalent cyclizine lactate concentration (for ampoules). Least squares linear regression analysis was performed and the results of the analyses are depicted in Tables 4.3 and 4.4. The calibration curve for the tablet assay had a slope of 0.2068, a y-intercept of 0.03012 and a correlation coefficient of 0.9993 and for the ampoule assay a slope of 0.1879, a y-intercept of -0.00366 and a correlation coefficient of 0.9994. Both calibration lines indicate the method has a linear response over the concentration ranges studied.

4.2.2.2 Precision and Accuracy

The results of within run precision determined as the relative standard deviation of peak height ratios of the calibration standards (n=6) are depicted in Tables 4.3 and 4.4 for the tablet and ampoule assays respectively. The precision of the assay in both circumstances is high as all relative standard deviations calculated were $\leq 5\%$. Accuracy was assessed by comparison of theoretical concentration to interpolated concentration of calibration standards and is reported as percent bias.

Table 4.3 Precision and Accuracy Data for Dosage Form Analysis of Valoid Tablets.

Calibration and Precision			Accuracy	
*CYC (ng/$\mu\ell$)	**PHR (n=6)	R.S.D. %	CYC Found ± S.D. (ng/$\mu\ell$)	% Bias
1	0.218	2.5	0.91 ± 0.0033	- 9.23
2	0.466	3.8	2.13 ± 0.11	+ 6.50
5	1.076	2.4	5.05 ± 0.12	+ 1.06
7	1.472	2.3	6.97 ± 0.16	- 0.38
9	1.887	1.6	8.98 ± 0.14	- 0.26

* Cyclizine hydrochloride concentration
** Peak height ratio of cyclizine hydrochloride/internal standard

Table 4.4 Precision and Accuracy Data for Dosage Form Analysis of Valoid Ampoules.

Calibration and Precision			Accuracy	
*CYC (ng/$\mu\ell$)	**PHR (n=6)	R.S.D. %	***CYC Found ± S.D. (ng/$\mu\ell$)	% Bias
1.18	0.216	1.78	1.17 ± 0.022	- 0.847
2.35	0.456	0.45	2.45 ± 0.0082	+ 4.26
5.89	1.075	1.15	5.74 ± 0.065	- 2.60
8.24	1.547	0.41	8.25 ± 0.034	+ 0.081
10.58	1.997	0.51	10.64 ± 0.054	+ 0.50

* Equivalent cyclizine lactate concentration
** Peak height ratio of cyclizine hydrochloride/internal standard
*** Equivalent cyclizine lactate concentration found

4.2.2.3 Dosage Form Analysis

Data obtained from the analysis of Valoid tablets and ampoules are tabulated in Table 4.5. The dosage forms analyzed were found to be within the limits specified in the U.S.P. for tablets and ampoules containing cyclizine hydrochloride or cyclizine lactate. Therefore, both the tablets and ampoules can be administered, with confidence, to volunteers participating in a bioavailability study, thereby ensuring that the correct dose had been given thereby removing a possible variable from the study.

Table 4.5 Results of Content Uniformity Analysis of Valoid Tablets and Ampoules.

Tablet	1	2	3	4	5	6	**Mean ± S.D.**
*Amt. mg (n=3) ± S.D.	50.46 ± 0.46	50.78 ± 0.13	49.54 ± 5.02	52.17 ± 0.0	51.28 ± 0.34	52.25 ± 4.78	51.08 ± 0.95
% Purity	100.92	101.56	99.08	104.34	102.56	104.49	102.16 ± 1.9
Ampoule	1	2	3	4	5	6	
*Amt. mg (n=3) ± S.D.	50.99 ± 0.33	51.96 ± 1.02	51.49 ± 0.37	51.52 ± 0.20	51.39 ± 0.49	52.02 ± 0.53	51.56 ± 0.35
% Purity	101.98	103.93	102.98	103.05	102.79	104.05	103.13 ± 0.70

* Amount of cyclizine hydrochloride per tablet or cyclizine lactate per 1ml ampoule

4.3 DISCUSSION

The U.S.P. method for the determination of cyclizine in dissolution and content uniformity studies is a complex and tedious procedure entailing back extraction procedures and analysis by ultra-violet spectrophotometry at 264nm. The preparation of samples by the method described and analysis by HPLC provides a simple, rapid, precise and accurate method for the assessment of dissolution of tablets containing cyclizine hydrochloride and for content uniformity determinations of tablets and ampoules containing cyclizine as the hydrochloride or lactate salts.

The results from dissolution studies on six commercially available tablets containing 50mg cyclizine hydrochloride suggest that rapid dissolution of the drug from the dosage form would occur and consequently no dissolution rate limited absorption *in vivo* would be expected. Content uniformity determinations indicate that both the tablets and ampoules may be administered to volunteers with the assurance that an accurate dose would be available for absorption from the gastro-intestinal tract, in the case of the tablets and that each subject would receive the same dose of cyclizine when the cyclizine lactate solution was injected intravenously.

CHAPTER 5

PHARMACOKINETIC AND BIOAVAILABILITY STUDIES ON CYCLIZINE

Cyclizine has been used successfully for the treatment of nausea and vomiting associated with motion sickness and for the treatment of vertigo in Ménière's Syndrome. The lack of sensitive and selective analytical methods has resulted in a paucity of information on the absorption and disposition pharmacokinetics of established drugs in human subjects despite their regular use over a number of years (161). Cyclizine is no exception in this regard. Pharmacokinetic literature has increased significantly in recent years (162) probably as a result of the advent of specific and sensitive analytical methods for the determination of drug and metabolite concentrations in biological fluids, the establishment of pharmacokinetic principles and concepts enabling the prediction of concentration-time profiles of drugs administered chronically and the realisation that pharmacological effects are related to drug or metabolite concentrations in plasma or serum (161).

Griffin and Baselt (46) reported C_{max}, t_{max} and half-life data following oral administration of 50mg cyclizine hydrochloride to a single male volunteer. In addition, they reported that urinary excretion after 24 hours of cyclizine was 0.01 percent of the administered dose and that norcyclizine was not isolated in neither serum nor urine. In another study (47), following oral administration of cyclizine to a single male volunteer, only C_{max} data were reported. Furthermore, both cyclizine and norcyclizine were isolated and concentrations of cyclizine were ten times higher than previously reported (46). Land *et al* (60) monitored cyclizine concentrations following intravenous administration of a 50mg dose to a single male volunteer. However, no pharmacokinetic information was reported. The methods used for the analysis of cyclizine in these studies seemed to show sufficient sensitivity for pharmacokinetic parameter elucidation, but, were applied to the analysis of samples obtained from pilot studies involving one volunteer, thus, precluding meaningful statistical assessment of the data. Therefore, the objective of the present study was to assess the bioavailability of cyclizine following single intravenous and oral doses and multiple oral dose administration of cyclizine to human volunteers. In addition, investigations to determine whether cyclizine exhibits dose-dependent pharmacokinetics were conducted.

Several important considerations are necessary when designing clinical pharmacokinetic studies, not the least of which are ethical in nature. These factors include site and frequency of sampling, considerations relating to choice of subjects and safety of personnel (163). It is important for investigators to realise that despite the need for a high degree of scientific skill, ethical concerns, particularly risk minimisation when performing research on human subjects must never be neglected.

Pharmacokinetic studies involve specimen collection (usually serum or plasma) and analysis of samples in attempts to establish a correlation between drug concentration in these fluids and therapeutic effects (91). However, meaningful results can only be obtained if the integrity of the sample can be guaranteed up to the point of analysis and if the sample collected is representative of the material to be analyzed. Considerations in this respect include accurate sampling times, labelling and storage.

5.1 PHARMACOKINETIC ANALYSIS OF DATA

5.1.1 Introduction

Pharmacokinetics is a the study of the rate processes of absorption, distribution, metabolism and excretion of drugs and their relationship to pharmacological, therapeutic or toxic responses in animals or humans (164). To describe complex biological systems, assumptions are made concerning the movement of drugs and mathematical terms are defined for the description of the time course of drugs and/or metabolites in accessible biological fluids such as blood or urine (165). Whilst these mathematical expressions have proven valuable to research and clinical use of a drug, a shortcoming is that the interpretation of *in vivo* data frequently leads to the use of compartment models which only approximate a biological system and reflect the reality of a drug-biological system interaction in a simplified way. Pharmacokinetic descriptions may make use of various non-compartmental estimates to further describe the time course of drug in the body. Parameters such as C_{max}, t_{max} and area under the plasma concentration time curve (AUC) are used to define the rate and extent of oral bioavailability. However, the use of statistical moments to determine mean residence time (MRT), mean absorption time (MAT) and area under the first moment curve (AUMC) provide useful pharmacokinetic information but are of limited use for the evaluation of comparative absorption rates in bioequivalence studies (166).

Pharmacokinetic parameters obtained following intravenous (IV) dosing provide the most meaningful data as a result of administration of drugs directly into the systemic circulation by-passing the absorption process and gut wall or hepatic first pass effects. In addition, a comparison of AUC values obtained from IV and oral (PO) dosing affords the researcher the opportunity of assessing the absolute bioavailability of the drug in question ensuring accurate determination of parameters such as clearance and volume of distribution that would otherwise be reported as a ratio of clearance or volume and bioavailability.

5.1.2 Compartmental Analysis

Pharmacokinetic compartmental models are hypothetical structures that can usually characterise the fate of a drug in the body following administration (68). The simplest compartment model describes the body as a single open compartment or 1BCM. More sophisticated models make use of a greater number of compartments to describe the disposition of a drug. The most commonly used multicompartment model is the open two-compartment model or 2BCM which describes drug disposition more reliably, but is susceptible to estimation problems as a result of multiple solutions, consequently leading to a lack of reliability of parameter estimates (167). Compartment models whether 1BCM, 2BCM or multicompartment systems are based on linear systems of differential equations. Elimination of the drug obeys, by implication, first order kinetics and total AUC should be a linear function of dose administered. In addition, models assume that changes in plasma levels of drug reflect proportional changes in tissue levels (165). A fundamental problem with model selection is that more than one model of comparable complexity may adequately describe the available data (168) and may be further confounded by "vanishing exponentials" (169). In addition, these models are used to deduce pharmacokinetic estimates that are not measured directly and may produce incorrect predictions of parameters despite providing a good fit to experimental data, as a result of indistinguishability (170). Despite regular use, doubt has been cast on the validity of using models that describe the body as a few kinetically homogenous compartments to describe the pharmacokinetics of a drug (171).

5.1.2.1 The One-Compartment Model

The one-compartment model (1BCM) represents the body as a single compartment with a specific volume (V_1). The drug is assumed to distribute rapidly between blood and other tissues in the body following entry into the body. The changes in plasma concentrations are assumed to reflect changes in tissue concentrations and elimination of the drug is assumed to occur as first order rate processes by oral, renal, biliary and metabolic pathways. The elimination rate constant (k_{el}) is the sum of the rate constants for individual elimination processes (172), and is proportional to the concentration of drug in the body fluids (173). In the case of oral dosing, first order absorption (k_a) and bioavailability (F) are important considerations for effective use of the model.

5.1.2.2 The Two-Compartment Model

Administered drugs invariably do not distribute instantly between the blood and other body fluids or tissues. Although, as for the one-compartment model, the compartments have no physiological or

anatomical reality, the two-compartment model (2BCM) represents the body as a pair of compartments in which the drug distributes between a central compartment (comprising body fluids and tissues in equilibrium with the circulatory system) and a peripheral compartment (comprising tissues and body fluids into which the drug distributes). The central compartment is readily accessible through blood sampling and drug levels should decline more rapidly during the distributive phase than post distributive phase, whilst levels in the peripheral compartment will increase to a maximum and then decline during distribution until an equilibrium is reached and distribution is terminated. The reversible distribution between the two compartments is dependent on blood flow, the drug's partition coefficient and tissue affinity for the drug (174). The introduction of a second compartment introduces various possibilities to the structure of the 2BCM. Three possibilities for the 2BCM exist;

a) a model in which elimination occurs exclusively from the central compartment,

b) a model in which elimination occurs only from the peripheral compartment, and

c) a model in which elimination occurs from both the central and peripheral compartments.

The 2BCM can be used for describing drug disposition following both intravenous and oral dosing providing absorption and bioavailability parameters are considered.

5.1.2.3 The Three Compartment Model

The three compartment model (3BCM) is essentially an extension of the 2BCM with an added deep tissue compartment. If drug disposition necessitates the use of a 3BCM to adequately explain processes within the body following drug administration then the model allows for rapid drug distribution to a highly perfused central compartment, less rapidly to a tissue compartment and slowly to a deep compartment. The third or deep compartment may represent tightly bound drug in the tissues. It is obvious that the introduction of a third compartment introduces further complexities in the description of drug disposition and as with the 2BCM, the 3BCM may have various structures, depending on the compartment(s) from which elimination may take place. In addition, these multicompartment models are rarely of pharmacological significance.

5.1.2.4 Which Model?

Compartmental analysis of experimental data should make use of the simplest model (least number of compartments) to describe that data. The number of compartments that are necessary to describe data may depend on (165);

a) route of administration,

b) absorption rate,

c) time and number of blood samples collected, and

d) sensitivity of the analytical method.

Consideration of these aspects is essential if the investigator is to use the correct model to determine realistic pharmacokinetic parameters of a drug. Too few samples collected in the elimination phase may suggest a third compartment or lack of sensitivity may preclude the observation of an additional compartment with a smaller first-order rate constant. Informative data ensuring correct model selection may be obtained by use of pre-experiment simulation with optimal sampling times (175).

5.1.3 Non-compartmental Analysis

5.1.3.1 **Bioavailability**

Bioavailability (F) may be defined as both the rate and relative amount (extent) of therapeutically active drug from an administered dosage form that appears in the systemic circulation (68,165). Intravenous bolus delivery allows the total dose to be made available to the body whilst oral dosing may allow only a fraction of the dose to reach the systemic circulation as a result of inefficient absorption, first-pass metabolism and/or dosage form effects. Consequently for intravenous administration, F is equal to unity and for oral administration F may vary between zero and unity depending on the extent of absorption via the gastrointestinal tract.

5.1.3.1.1 Absolute Bioavailability

Absolute bioavailability is assessed by comparison of area under the drug concentration-time curve (AUC) values obtained following oral administration (AUC_{PO}) and that obtained following intravenous administration (AUC_{IV}) of a drug (68,165). Absolute bioavailability is calculated using equation 5.1. The intravenous route is the reference standard since, by definition, an intravenous dose is completely bioavailable (*i.e.* 100%). The measurement of absolute bioavailability attempts to determine the effects of body functions and processes on drug absorption.

$$F = \frac{AUC_{PO} \times DOSE_{IV}}{AUC_{IV} \times DOSE_{PO}} \qquad \text{Eq. 5.1}$$

where F = absolute bioavailability.

5.1.3.1.2 Relative Bioavailability

The relative bioavailability of a drug product measures the absorption characteristics of one pharmaceutical equivalent compared with another and essentially determines formulation effects on the rate and extent of drug absorption. Relative bioavailability is assessed by comparison of AUC values obtained following oral administration of a reference formulation and a test formulation and is calculated using equation 5.2.

$$F = \frac{AUC_{test} \times DOSE_{ref}}{AUC_{ref} \times DOSE_{test}} \qquad \text{Eq. 5.2}$$

where F = relative bioavailability.

5.1.3.2 **Area Under Curve (AUC)**

The area under the drug concentration-time curve (AUC) is a measure of the quantity of drug that reaches the systemic circulation and is used to assess bioavailability. AUC is frequently calculated by the linear trapezoidal method:

$$AUC = \sum \left(\frac{C_n + C_{n-1}}{2}\right) \times (t_n - t_{n-1}) \qquad \text{Eq. 5.3}$$

where C_n = plasma concentration at time (t_n).

An inherent error of the linear trapezoidal method is the under-estimation of area during the absorption phase and an over estimation in the post-absorption phase (176).

A modification of the linear trapezoidal method is the log trapezoidal method in which the concentration in the plasma is assumed to vary linearly within a sample interval on a semilogarithmic scale. The use of equation 5.4 in pharmacokinetics is most appropriate when the data in the post-absorption phase appear to decline exponentially.

$$AUC = \frac{C_n - C_{n-1}}{\frac{1}{t_n - t_{n-1}} \times \ln \frac{C_n}{C_{n-1}}} \qquad \text{Eq. 5.4}$$

The use of the log trapezoidal method is not without limitations as large errors may be introduced when this method is used in the absorption phase, near a peak or in a polyexponential post-absorption phase. In addition, if a concentration value is zero or two concentrations are equal, this method

cannot be used. Nevertheless, despite these disadvantages the log trapezoidal method may be used alone or in combination with other methods to provide appropriate solutions to area calculations (177).

Due to the inherent simplicity of the trapezoidal methods they are a sensible choice for AUC calculation. However, the AUC calculated is only the area to the last measurable concentration and the rest or remaining area must be determined to obtain the total area under the curve from zero time to infinity (AUC^{∞}). The assumption is made that plasma concentrations decrease monoexponentially with time after the last data point. The remaining area is calculated using equation 5.5 and the total area under the curve using equation 5.6.

$$AUC^{n\rightarrow\infty} = \frac{C_n}{k_{el}} \qquad \text{Eq. 5.5}$$

where C_n = the last measurable concentration, and
k_{el} = the slope of the terminal portion of a semilogarithmic concentration-time plot.

The validity of using the last measured concentration (C_n) has recently been questioned by Purves (178) who stated that using the fitted value (C_z) at the last time point would reduce bias when using equation 5.5.

$$AUC^{0\rightarrow\infty} = AUC^{0\rightarrow n} + AUC^{n\rightarrow\infty} \qquad \text{Eq. 5.6}$$

The usefulness of AUC is not limited to indication of the extent of drug absorption only. Evaluation of AUC values obtained following oral administration of single doses of increasing dose size give an indication as to whether a drug may exhibit dose dependency or undergo first-pass metabolism (68).

Following multiple dosing, AUC values obtained during one dosing interval ($AUC^{\tau n\rightarrow\tau n+1}$) at steady state should be equivalent to AUC^{∞} after a single dose of the drug. A comparison of these AUC values would indicate whether enzyme saturation or induction occurs during multiple dosing of a drug. The relationship between AUC obtained after single or multiple dosing to steady state and the dosing interval, enables the calculation of an average steady concentration (C^{SS}_{av}) that will be reached during multiple dosing. This prediction is only valid in the absence of dose dependency, first-pass metabolism and enzyme induction or inhibition.

5.1.3.3 Rate of Absorption

Drug absorption is a complex procedure involving many individual rate processes, including dissolution rate, gastric motility, and drug transport across membranes. The absorption rate of any drug is the overall result of these processes. Consequently variations in absorption rate of a drug via a particular route of administration may vary and may be explained by the fact that the measured parameter is an "apparent" absorption rate constant (68). Calculation of absorption rates based on the assumption that drugs follow compartmental disposition have been published and include the Wagner-Nelson (179) and Loo-Riegelman (180) methods. Tucker (181), has reviewed the use of methods for the determination of absorption rates based on reading information directly from the blood profiles or use of compartment models if these best explain absorption rates and drug disposition.

In bioavailability and bioequivalence studies, simple but crude measures of rate of absorption are the maximum concentration (C_{max}) and the time to reach maximum concentration (t_{max}). Endrenyi *et al* (182) have recently suggested that the ratio of C_{max} and AUC be used in preference to C_{max} as a measure of absorption rate in bioequivalence studies as the ratio is independent of extent of absorption and is therefore an unambiguous measure of absorption rate. In addition, presentation of this ratio as a percentage enables a simple and rapid assessment of differences in bioequivalence studies.

5.1.3.4 Elimination Rate (λ_Z), Elimination Half-Life ($t_{½}$) and Clearance (Cl_{TOT})

Elimination of most drugs from the body follows a first-order process. If a drug follows one compartment kinetics the elimination rate constant (k_{el}) describes the total removal of drug from the body as a result of biotransformation and excretion. The elimination rate constant represents the sum of each of the individual rate processes (equation 5.7) and is referred to as the overall elimination rate constant (K).

$$K = k_m + k_e \qquad \text{Eq. 5.7}$$

where k_m = first-order rate process of metabolism, and
k_e = first-order rate process of excretion.

If a drug follows two compartment kinetics the hybrid elimination rate constant (ß) is used to describes the terminal elimination rate and represents drug elimination from the body after equilibrium has been established.

In an effort to eliminate confusion with symbols in pharmacokinetics Aronson *et al* (183) have suggested a standardization of symbols and suggest that the terminal or slowest exponential term be assigned the rate constant λ_Z and is calculated from the terminal slope of a semilog plot of serum concentration versus time. The elimination rate constant can also be determined from the slope of a plot of urinary excretion rate data versus midpoint time of the collection period on a semilogarithmic scale based on the assumption that excretion is a first order process.

The terminal half-life ($t_{½}$) of a drug is a function of its elimination and distribution and can be estimated from the terminal phase (λ_Z) of a semilogarithmic plot of a serum concentration versus time profile or by use of equation 5.8.

$$t_{\frac{1}{2}} = \frac{0.693}{\lambda_Z} \qquad \text{Eq. 5.8}$$

Practically, a knowledge of the half-life of a drug, in conjunction with other pharmacokinetic parameters, enables the pharmacokineticist to design effective dosage regimens and predict times to reach steady state (184) if the drug follows monoexponential disposition kinetics (185). In addition, knowledge of the half-life allows the analyst to determine the time necessary (4-6 half-lives) to collect and analyze blood and urine samples for effective characterization of the pharmacokinetics of a drug compound (123).

Organ clearance is in effect the ratio of elimination rate to drug concentration in the blood entering the organ (172). Of all organs involved in clearance of drug, the most accessible to pharmaceutical scientists is the kidney. Renal clearance (Cl_R) can be determined if intact drug is excreted in the urine to some extent (172). Cl_R is estimated by measuring drug concentration in a volume of urine collected for known periods of time following administration using equation 5.9.

$$Cl_R = \frac{\frac{dA_e}{dt}}{C_{p\ mid\text{-}point}} \qquad \text{Eq. 5.9}$$

where dA_e/dt = excretion rate, and
$C_{p\ mid\text{-}point}$ = plasma concentration at mid-point urine collection period.

Total body clearance (Cl_{TOT}) is a measure of removal of drug from the body and is defined as the volume of distribution completely cleared of drug in a specified time. CL_{TOT} takes both $t_{½}$ and apparent volume of distribution into account and is thus a more useful measure of drug removal from

a system than elimination half-life alone (186). Clearance is the sum of all individual organ clearances that contribute to the overall elimination of a drug. The difference between Cl_{TOT} and Cl_R produces a value that combines all other routes of excretion, inclusive of metabolism and, is extrarenal clearance (CL_{NR}). This relationship is depicted in equation 5.10.

$$Cl_{TOT} = Cl_R + Cl_{NR} \qquad \text{Eq. 5.10}$$

where Cl_{TOT} = total clearance,
Cl_R = renal clearance, and
Cl_{NR} = extrarenal clearance.

Cl_{TOT} can only be calculated from serum concentration data obtained following intravenous dosing as it is the ratio of overall elimination rate to drug concentration. The integral of elimination rate is equivalent to the total amount of drug excreted which, in the case of intravenous administration is the dose. Thus, Cl_{TOT} can be calculated, in intravenous studies, using equation 5.11.

$$Cl_{TOT} = \frac{DOSE}{AUC^{0\rightarrow\infty}} \qquad \text{Eq. 5.11}$$

For studies in which drugs are administered via the oral route the estimate of Cl_{TOT} may not be accurate as the total dose administered may not be absorbed. The estimate is termed "apparent clearance" and should be reported as a ratio of clearance and bioavailability (F). If F is known, then an accurate estimate of CL_{TOT} may be obtained using equation 5.12.

$$Cl_{TOT} = \frac{F \times DOSE}{AUC^{0\rightarrow\infty}} \qquad \text{Eq. 5.12}$$

where F = absolute bioavailability.

5.1.3.5 **Volume of Distribution**

The volume of distribution (V_d) is a hypothetical volume of fluid into which the total amount of administered drug would have to be dissolved to achieve the same concentration as that found in the blood. It is essentially a proportionality constant relating the amount of drug in the body to the concentration of drug in the sampled fluid (*viz.* blood, serum or plasma).

Several methods may be used to estimate the apparent V_d. Each method requires administration of an intravenous bolus dose in order for the amount reaching the systemic circulation to be equivalent to the dose and to be a known quantity. Gibaldi and Perrier (172) have reviewed methods for the estimation of the various volumes of distribution.

Extrapolation of data to estimate the initial drug concentration immediately following an intravenous dose, if instantaneous distribution of drug between plasma and tissues occurs, enables the calculation of a volume of distribution often termed the extrapolated volume of distribution ($V_{extrapolated}$) using equation 5.13. Application of this equation following oral drug administration is not advocated.

$$V_{extrap} = \frac{DOSE}{C_0} \qquad \text{Eq. 5.13}$$

where C_0 = initial drug concentration.

A further shortcoming of the use of equation 5.13 is that, in reality, few drugs distribute instantaneously throughout the body space and therefore, serum drug concentration time profiles are best described by multiexponential equations which may result in overestimation of the apparent volume of distribution. Therefore, the use of equation 5.13 is limited to situations in which a monexponential function would best describe serum drug concentration profiles in the body.

An expression of volume of distribution that is more meaningful is V_{area} or V_{β}. Aronson *et al* (183) have suggested the use of V_Z to describe the volume of distribution derived from the relationship between AUC^{∞} and the intravenous dose of the drug. This relationship is depicted in equation 5.14 and relates drug concentration in the plasma to the total amount of drug in the body during the terminal exponential phase where elimination occurs from the sampled compartment.

$$V_Z = \frac{DOSE}{\lambda_Z \times AUC_0^{\infty}} \qquad \text{Eq. 5.14}$$

where λ_Z = terminal slope of curve of linear multicompartment system.

Following oral dosing equation 5.14 may be modified by incorporation of the term absolute bioavailability thus taking the form:

$$V_Z = \frac{F \times DOSE}{\lambda_Z \times AUC_0^\infty} \qquad \text{Eq. 5.15}$$

where F= absolute bioavailability.

The most meaningful volume term that describes the apparent volume of distribution in a multicompartment system is the volume of distribution at steady-state (V_{ss}) (172). V_{ss} defines the relationship between amount of drug in the body and concentration, at steady-state, during multiple dosing and is independent of drug elimination. The volume at steady-state is a function of the transfer constants between the central and tissue compartments and can be calculated using equation 5.16.

$$V_{ss} = V_c + \frac{k_{12}}{k_{21}} V_c \qquad \text{Eq. 5.16}$$

where V_c = volume of the central compartment, and,
k_{12}, k_{21} = transfer rate constants from and to the central compartment respectively.

However, this calculation requires curve fitting or an assumption of a model to describe the pharmacokinetics of the drug. A more general, model independent approach has been described (191) and is depicted in equation 5.17. The use of this method does not require the assumption of a compartment model or curve-fitting procedures.

$$V_{ss} = \frac{D\ [AUMC^\infty]}{[AUC^\infty]^2} \qquad \text{Eq. 5.17}$$

where D = dose,
$AUMC^\infty$ = area under the first moment curve to infinity, and
AUC^∞ = area under the curve to infinity.

Perrier and Mayersohn (187) adapted the determination of V_{ss} described in equation 5.16 to encompass any mode of administration and may be calculated using equation 5.18.

$$V_{ss} = FD \frac{[AUMC^\infty]}{[AUC^\infty]^2} - \frac{FD}{k_a\ AUC^\infty} \qquad \text{Eq. 5.18}$$

where F = bioavailability, and,
k_a = first order absorption rate.

5.1.3.6 Statistical Moment Analysis

The use of statistical moments in pharmacokinetics was first described by Yamaoka *et al* (188) and Cutler (189). The moment concept was later clarified by Riegelman and Collier (190). Statistical moment theory considers the time course of serum concentration after a single dose of drug to be a statistical distribution curve (188). As the statistical moments are dependent only on the observed concentration time data they are independent of any compartmental model. The AUC^{∞} represents the zero moment, and has been discussed in § 5.2.3.2. The first normal moment is represented by the area under the first moment curve ($AUMC^{\infty}$) and is the area under the curve of the product of serum concentration (C_p) and time (t) (191). Mean residence time (MRT) as defined by Openheimer *et al* (192) can be determined using equation 5.19.

$$MRT = \frac{\int_0^{\infty} t\, C_p\, dt}{\int_0^{\infty} C_p\, dt} = \frac{AUMC^{\infty}}{AUC^{\infty}} \qquad \text{Eq. 5.19}$$

The MRT estimated using equation 5.19 is based on the assumption that clearance or volume of distribution are constant, linear and that drug elimination occurs as a linear process from the compartment where sampling takes place (193). The MRT is defined as the mean time for intact drug molecules to transit through the body and is a description of all kinetic processes including release from the dosage form and absorption into the body. Therefore, for a drug administered orally other than the intravenous route, the ratio of $AUMC^{\infty}$ to AUC^{∞} is equal to the sum of mean times for individual processes such as dissolution (MDT) and absorption (MAT) as well as MRT (190).

The exact value of MAT can be estimated by subtraction of the MRT obtained following an intravenous dose (MRT_{IV}) of drug from that obtained following an oral dose (MRT_{PO}) of drug as described in equation 5.20.

$$MAT = MRT_{PO} - MRT_{IV} \qquad \text{Eq. 5.20}$$

Collier (194) has proposed that a term describing the sum of individual mean times (SMT) be used to describe all factors, including mean dissolution, absorption and residence times, that contribute to the ratio $AUMC^{\infty}$ to AUC^{∞} for drugs not administered by an intravenous bolus dose. Recently Karol (195) has emphasised that in reality the ratio $AUMC^{\infty}/AUC^{\infty}$ can only estimate MRT for a drug that is administered as an intravenous bolus dose and that when administered via the oral route the term is the sum of MRT and the absorption time (MAT).

5.1.4 Multiple Dose Pharmacokinetics

Most drug therapy involves repeated administration of a drug in separate fixed doses for variable periods. When administered as a series of fixed multiple doses, accumulation of drug in the plasma occurs until it reaches a steady state at which time peak (C^{ss}_{max}) and trough (C^{ss}_{min}) concentrations remain constant from dose to dose. The time required to reach steady state is dependent on the elimination half-life of the drug and is independent of the size of dose, length of dosing interval and number of doses (165). Several concepts with regard to multiple dose data processing are that all dose sizes should be equal, the dosing intervals should be the same and all pharmacokinetic parameters must remain constant throughout therapy (68).

Bioequivalence assessments following multiple dosing require characterisation of absorption rates. Schulz and Steinijans (196) have suggested that peak-trough fluctuations reported as a percentage difference between maximum and minimum concentrations with respect to the average concentration is a superior method of assessing absorption rate than percent swing in which peak-trough differences are related to the minimum concentration and percent AUC-fluctuation as this steady state rate parameter eliminates individual clearance differences producing an independent rate characteristic. The percent peak-trough fluctuation may be calculated using equation 5.21.

$$\% \; PTF = \frac{100 \; (C^{ss}_{max} - C^{ss}_{min})}{C^{ss}_{av}} \qquad \text{Eq. 5.21}$$

Obvious advantages of using multiple dosing in bioequivalence assessment are that the patients act as their own control, and serum concentrations are higher and consequently can be more accurately assayed ensuring reliable results (165). In addition higher serum concentrations preclude the need for highly sensitive and costly detection systems.

Several additional pharmacokinetic parameters may be determined following multiple dosing of a drug. The relationship between AUC^{∞} and dosing interval allows the prediction of the average steady state concentration (C^{ss}_{av}) (equation 5.22). Further, area under the curve to infinity after a single dose is equivalent to area under the curve for one dosing interval if the drug follows linear kinetics.

$$C^{ss}_{av} = \frac{AUC^{\infty}_{0}}{\tau} = \frac{AUC^{\tau \; n+1}_{\tau \; n}}{\tau} \qquad \text{Eq. 5.22}$$

where τ = dosing interval.

It is evident therefore that the magnitude of C^{ss}_{av} is proportional to the dose size and extent of absorption. In addition, C^{ss}_{av} may be calculated using equation 5.23.

$$C_{av}^{ss} = \frac{F \times DOSE}{Cl_{TOT}\,\tau} \qquad \text{Eq. 5.23}$$

where F = absolute bioavailability, and,
Cl_{TOT} = total clearance.

Total clearance may be calculated from multiple dose studies if a drug obeys linear kinetics as a result of the equivalence of AUC^{∞} and the area under the curve during one dosing interval by substitution of $AUC^{\tau n \rightarrow \tau n+1}$ into equation 5.11.

The C^{ss}_{av} concentration does not give information regarding fluctuations in concentrations that occur at steady state (C^{ss}_{max} and C^{ss}_{min}). The maximum plasma value is important in terms of determining drug safety and is a good indication of accumulation. The minimum and maximum plasma concentrations may be estimated using equations 5.24 and 5.25 for C^{ss}_{min} and C^{ss}_{max} respectively.

$$C_{min}^{ss} = \frac{F \times DOSE}{V} \times \frac{e^{-\lambda_z \tau}}{1 - e^{-\lambda_z \tau}} \qquad \text{Eq. 5.24}$$

where F = absolute bioavailability,
V = volume of distribution,
λ_Z = terminal elimination rate constant, and,
τ = dosing interval.

$$C_{max}^{ss} = \frac{F \times DOSE}{V} \times \frac{1}{1 - e^{-\lambda_z \tau}} \qquad \text{Eq. 5.25}$$

where F = absolute bioavailability,
V = volume of distribution,
λ_Z = terminal elimination rate constant, and,
τ = dosing interval.

5.2 ABSORPTION AND DISPOSITION OF CYCLIZINE IN HUMANS

5.2.1 Study Design

5.2.1.1 Study Population

Male volunteers that were found to be in good health, determined on the basis of physical examination, haematological tests and urinalysis were used in the trials after they had given their informed consent. The haematological tests conducted included glucose, urea, serum creatinine, total bilirubin, total protein, ESR, full blood count, and SGOT and SGPT. Urinalysis included pH, glucose, protein and blood in addition to a microscopic examination. Subjects were excluded from participation in the study if they had a history of liver disease, allergy to cyclizine or related compounds, asthma or related disorders. In addition, subjects that used medication regularly, abused alcohol, were smokers or had been treated with a compound known to have potential toxicity to one of the major organs within three months preceding the study were not considered as suitable candidates for the study. The demographic data of the subjects and the studies in which they participated are tabulated in Table 5.1.

Table 5.1 Demographic Data of Volunteers Used in Bioavailability and Pharmacokinetic Studies.

Subject No.	Name	Age (yrs)	Height (m)	Mass (kg)	Phases
1	A.B.	20	1.76	76	All
2	P.S.	20	1.93	99	1,2,3
3	M.B.	19	1.85	73	All
4	G.W.	18	1.84	83	All
5	S.S.	19	1.88	81	1,2,3
6	D.H.	19	1.74	68	1,2,3

5.2.1.2 Study Performance

The clinical phases of the bioavailability and pharmacokinetic studies were conducted in 5 phases of which phases 1, 2 and 3 were conducted in a parallel fashion and phases 4 and 5 in a two-way cross-over fashion. Of the subjects selected for the bioavailability studies, subjects 1, 3, and 4 participated in all five phases.

5.2.1.3 Intravenous Study (Phase 1)

Subjects were asked to refrain from taking medication for at least a week prior to the commencement of the study. In addition, participants were asked to abstain from ingestion of alcohol and caffeine containing food and beverages for at least 48 hours, and to avoid strenuous exercise for 24 hours prior to the commencement of each phase of the studies.

Following an overnight fast, subjects were asked to report to the trial centre at 7.00am on the morning of the trial. On arrival, a urine specimen was collected for analysis and preparation of standards. An indwelling, winged infusion set (Terumo Corporation, Tokyo, Japan) was inserted into a suitable arm vein and a blood sample (blank) withdrawn. The catheter was secured and retained in position for the duration of the trial. After removal of the blank sample, the catheter was flushed at 5 minute intervals for 15 minutes with a 50 IU/ml heparin solution in normal saline. The catheter was flushed every hour and immediately after withdrawal of a blood sample. The practice of flushing the catheter regularly helped reduce the number of blockages throughout the trial period, thereby promoting efficiency with respect to sample withdrawal.

Subjects were administered 0.5ml Valoid injection (Wellcome (Pty) Limited, Johannesburg, Republic of South Africa) containing 25mg cyclizine lactate (18.682mg cyclizine base equivalent) administered as a single intravenous bolus dose over 2-3 minutes together with 250ml of water and were required to remain supine for 4 hours after administration of the dose and then remain sedentary for 6 hours after dosing. Thereafter, subjects were allowed to be ambulatory, but were not permitted to partake in any strenuous activity.

Volunteers received a light breakfast and a standard low-fat lunch 2 and 5 hours respectively after adminstration of the dose. Breakfast consisted of 30g cereal, one teaspoon of sugar, 100ml of skimmed milk, four slices of lightly buttered toast with jam and 250ml of orange juice and the lunch of chicken, vegetables, green salad, fruit salad and 250ml of orange juice. Volunteers were permitted to eat an evening meal of their own choice and these meals were therefore not standardised.

5.2.1.4 Oral Study (Phase 2)

Trial volunteers were asked to follow the same procedures as described in § 5.1.1.3 and after administration of one 50mg Valoid tablet (Wellcome (Pty) Limited, Johannesburg, Republic of South Africa) containing 50mg cyclizine hydrochloride equivalent to 43.989mg cyclizine base as a single oral dose were subject to the same restrictions with respect to ambulation.

5.2.1.5 Multiple Dose Study (Phase 3)

All subjects participating in phase 3 of the study were asked to refrain from taking medication for at least a week prior to commencement and for the duration of the study. In addition, ingestion of alcohol and caffeine containing beverages was prohibited for the duration of the trial. Subjects were also asked to avoid participation in strenuous exercise. Limitations with respect to diet were not imposed, however, subjects were asked to limit fat content of all meals. At the commencement of the study a blood sample (blank) was withdrawn and each subject was administered one 50mg Valoid tablet with 250ml water. Thereafter, volunteers returned to the trial centre every 8 hours to receive subsequent doses. A trough blood sample was collected before dose 15 (112 hours) by venipuncture. Subjects were asked to fast prior to administration of the sixteenth dose (120 hours). On arrival at the trial centre a catheter was inserted into an arm vein as previously described (§ 5.1.1.3) and a blood sample (trough) withdrawn after which each subject received a 50mg cyclizine hydrochloride tablet with 250ml water. A light breakfast and standard lunch were served at 2 and 5 hours respectively after that dose.

5.2.1.6 Dose-Dependency Study (Phases 4 and 5)

Subjects 1, 3 and 4 only participated in these phases of the study and were subject to the restrictions imposed on participation in these studies (§ 5.1.1.3). Subjects received either two 50mg Valoid tablets (87.979mg cyclizine base equivalent) or three 50mg Valoid tablets (131.968mg cyclizine base equivalent) as single oral doses. Blood samples were withdrawn via a catheter that had been inserted as previously described (§ 5.1.1.3)

5.2.1.7 Sample Collection and Storage for Analysis

Ten millilitre (10ml) blood samples were withdrawn at the following times after dosing:-

Phase 1:- 0, 0.08, 0.17, 0.33, 0.50, 0.67, 0.83, 1.0, 1.25, 1.50, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hours.

Phase 2:- 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hours.

Phase 3:- Samples were taken before the first, fifteenth and sixteenth dose and then as follows after the sixteenth dose, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 24.0, 30.0, 36.0, 48.0, 60.0, and 72.0 hours.

Phase 4 & 5:- 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, and 24.0 hours.

Duplicate blood samples were withdrawn at random times, in all phases, of the study, for use as *ex vivo* controls during analysis of the samples. Serum was collected from whole blood samples that had been allowed to clot for 30 minutes and had been centrifuged at 1600xg for 20 minutes. The serum was transferred to a clean-test tube and a 1ml aliquot of serum pipetted into a second clean test-tube for the initial analysis. The surplus serum was retained for any repeat analysis of samples that may have been necessary. All samples were stored at -15°C until analyzed.

Urine samples were only collected during phase 1 and 2 of the studies. Urine was collected prior to dosing and collection periods for urine were 0-2, 2-4, 4-8, 8-12, 12-24, and 24-36 hours after adminstration of cyclizine. Urine samples produced during and at the termination of the collection period were pooled. The volume and pH of the pooled urine samples were recorded and an aliquot stored, in duplicate, in clean test-tubes at -15°C until analyzed.

5.2.1.4 Sample Analysis

Concentrations of both norcyclizine and cyclizine in serum and urine were determined by solid-phase extraction and HPLC with electrochemical detection as described in § 3.3.1.1 and § 3.1.7 respectively. Serum samples were analyzed using a calibration range from 0-100ng/ml for both cyclizine and norcyclizine. A calibration range from 0-400ng/ml was employed for the analysis of norcyclizine and cyclizine in urine samples. Serum and urine samples with concentrations above the calibration range were diluted accordingly with blank serum or urine and re-analyzed.

Serum and urine calibration standards were prepared prior to the commencement of analysis of samples and stored at -15°C until used for analysis. The results of stability studies (§ 2.3.2.1 and § 2.3.2.2) indicate that the samples would be stable for 1 month at this storage temperature. The advantage of preparing standards and storing them is that samples to be analyzed can be prepared in advance thereby eliminating lengthy preparation of standards on the day of analysis.

5.3 RESULTS AND DISCUSSION

5.3.1 Intravenous Studies (Phase 1)

Serum and urine samples obtained following the IV administration of 25mg cyclizine lactate (18.682mg cyclizine base) as a bolus dose to each of six volunteers, were extracted and analyzed by HPLC (§ 3.1.7 and § 3.3.1.1). Semilogarithmic serum concentration time plots for each volunteer are depicted in Figure 5.1 and cumulative urinary excretion plots (36 hours) for both cyclizine (CYC)

and its demethylated metabolite, norcyclizine (NCYC) are shown in Figure 5.2. It is interesting to note that the main metabolite, norcyclizine was isolated in urine samples but not serum samples following the intravenous dosing of cyclizine lactate.

The pharmacokinetic parameters for cyclizine obtained from analysis of these serum and urine data are summarised in Table 5.2.

Table 5.2 Cyclizine Pharmacokinetics for Subjects 1 to 6 Following Administration of an Intravenous Bolus Dose of 0.5ml of a Cyclizine Lactate Solution (50mg/ml).

SUB	$AUC^{0\rightarrow\infty}$ $\mu g/\ell.hr$	$AUMC^{0\rightarrow\infty}$ $\mu g/\ell.hr^2$	MRT hr	λ_z hr^{-1}	$t_{½}$ hr	V_{ss} ℓ/kg	V_z ℓ/kg
1	237.63	3603.20	15.16	0.0492	16.52	15.68	21.04
2	259.00	4047.94	15.62	0.0510	13.58	11.38	14.29
3	324.89	5081.46	15.64	0.0562	12.32	12.32	14.01
4	255.97	1579.93	12.57	0.0658	10.53	11.06	13.37
5	263.02	5193.58	19.74	0.0433	16.00	17.32	20.23
6	300.65	4068.53	14.03	0.0568	12.21	12.82	16.10
Mean	275.24	4068.53	15.76	0.0531	13.79	13.57	16.70
± S.D.	29.34	1337.93	2.79	0.0081	2.48	2.54	3.29

The area under the curve was calculated as described in § 5.2.3.2 and area under the first moment curve as described in § 5.2.3.6. The mean residence time (MRT) was estimated from these data using equation 5.19. The terminal rate constant, (λ_z), was calculated by linear regression of the terminal slope of a semilog plot of serum concentration versus time. From these data, the elimination half-life ($t_{½}$) was calculated using equation 5.8. The mean value of 13.79 ± 2.48 hours obtained for $t_{½}$ is shorter than that of 24 hours (46) obtained following a single oral dose of cyclizine hydrochloride to a single volunteer. In addition, it is shorter than the 22 hours calculated following administration of an oral dose to a single volunteer (47). V_z and V_{ss} were calculated using equations 5.14 and 5.17 respectively. V_z usually provides a close approximation of V_{ss} (172). However, for benzylpenicillin in humans the value of 26ℓ for V_z was significantly larger than the calculated V_{ss} of 15ℓ (197). Gibaldi and Perrier (172) suggest that this difference is indicative that the disposition of a drug is better described by a system in which drug distribution occurs over a finite period and the body does not behave as a single compartment. The mean value of 16.70 ± 3.29 ℓ/kg for V_z clearly indicates extensive distribution of CYC to the "deep tissue" (68). A high value for V_z is expected since experiments in which CYC was administered to male rats resulted in concentrations of CYC in the lung, spleen, liver and kidney 20 to 110 fold higher than those in plasma (43).

Total (Cl_{TOT}), renal (Cl_R) and nonrenal clearance (Cl_{NR}) data are summarised in Table 5.3. Cl_{TOT} was calculated using equation 5.11. Cl_R was calculated for each collection period using equation 5.9 and the value reported is the mean renal clearance for each subject. In addition, the percent of the dose excreted as unchanged drug or eliminated as the metabolite, norcyclizine is reported as a percentage of the administered dose excreted as cyclizine.

Table 5.3 Clearance and Urinary Excretion Data for Subjects 1 to 6 Following Administration of an Intravenous Bolus Dose of 0.5ml of a Cyclizine Lactate Solution (50mg/ml).

SUB	Cl_{TOT} ℓ/hr/kg	Cl_R ℓ/hr/kg	*Cl_{NR} ℓ/hr/kg	**NCYC %	*CYC %
1	1.034	0.00576	1.028	1.03	0.53
2	0.729	0.00877	0.720	1.19	0.10
3	0.788	0.00258	0.785	0.71	0.32
4	0.879	0.00565	0.873	0.83	0.46
5	0.877	0.00427	0.873	0.59	0.41
6	0.914	0.00528	0.909	0.73	0.57
Mean ± S.D.	0.865 0.0966	0.00539 0.00186	0.859 0.0969	0.85 0.20	0.39 0.16

* $Cl_{NR} = Cl_{TOT} - Cl_R$

** Percent of dose of norcyclizine excreted in urine corrected for cyclizine

* Percent of dose of cyclizine excreted in urine

Cl_{TOT} and CL_R were found to be 0.865 ± 0.097 and 0.0054 ± 0.0019 ℓ/hr/kg respectively following intravenous administration of CYC. The Cl_R for parent drug formed only a small proportion of Cl_{TOT} for that drug indicating that urinary excretion of parent drug is not a major route of elimination for CYC. These findings are confirmed by the fact that less than one percent of the dose was excreted as unchanged drug in the urine (0.39 ± 0.16%). This value is in close agreement with the findings of other authors (46,47,74,80). Metabolic inactivation of CYC, primarily by demethylation (43) to form NCYC or by glucuronidation (73) in the liver, is an indication that extrarenal clearance accounts for most of the removal of drug from the body. In addition, the presence of norcyclizine in urine further supports alternative clearance mechanisms.

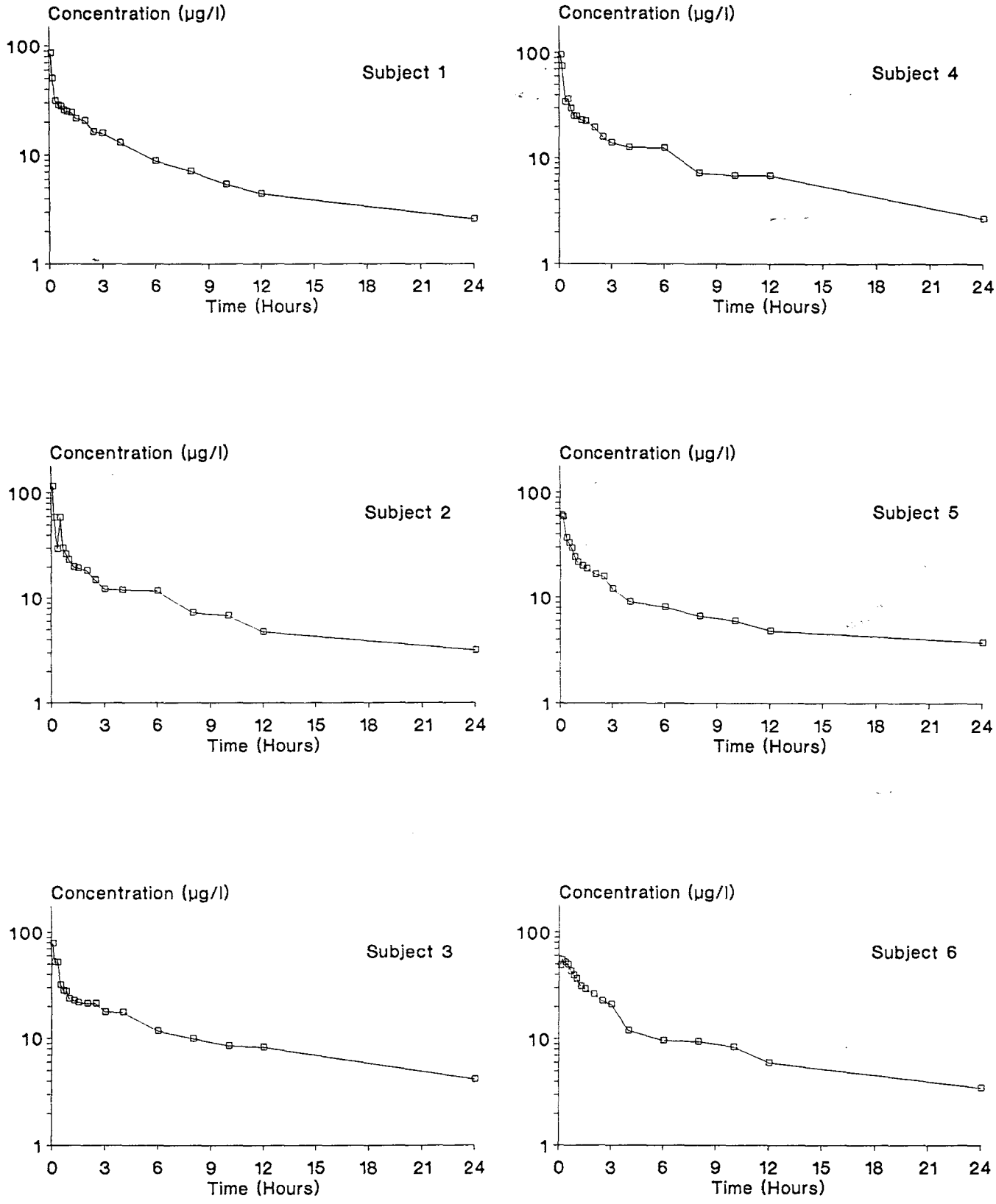


Figure 5.1 Semilogarithmic serum concentration time profiles for subjects 1 to 6 showing cyclizine (□) following administration of a single (0.5ml) intravenous bolus dose of cyclizine lactate solution.

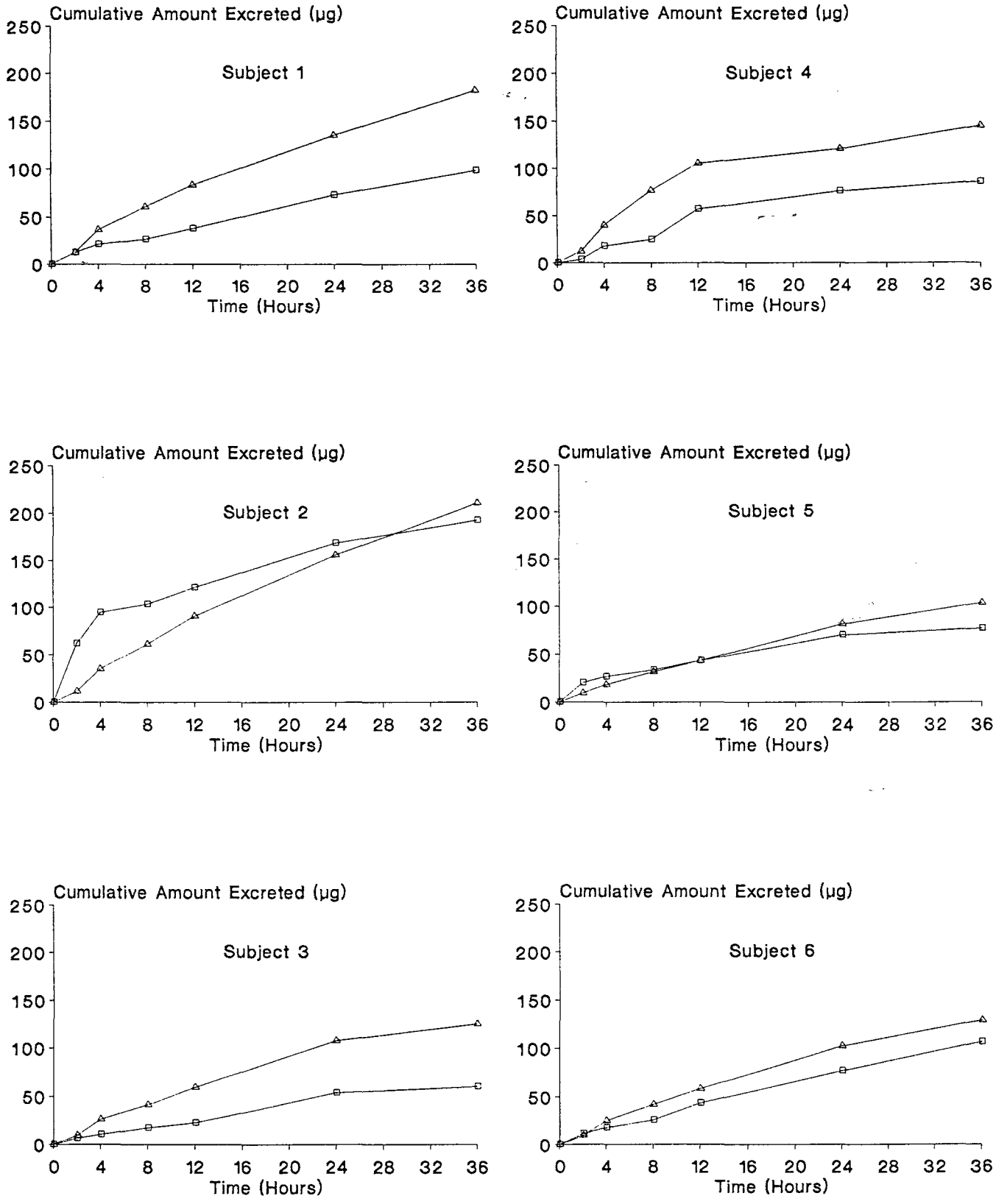


Figure 5.2 Cumulative urinary excretion profiles for subjects 1 to 6 showing both norcyclizine (△) and cyclizine (□) following administration of a single (0.5ml) intravenous bolus dose of cyclizine lactate solution.

5.3.2 Oral Studies (Phases 2, 4, and 5)

Serum and urine samples obtained from subjects 1 to 6 following administration of a single 50mg cyclizine hydrochloride tablet were extracted and analyzed by HPLC (§ 3.1.7 and § 3.3.1.1) and semilogarithmic plots of serum concentration versus time for both norcyclizine and cyclizine are shown in Figure 5.3. Cumulative urinary excretion profiles for subjects 1 to 6 are depicted in Figure 5.4. Serum samples only were collected from only 3 volunteers in phases 4 and 5 of the study. These subjects each received two or three 50mg cyclizine hydrochloride tablets and serum samples were collected, extracted and analyzed by HPLC.

5.3.2.1 **Phase 2**

Semilogarithmic plots of serum concentration versus time profiles for these subjects are depicted in Figure 5.3. A comparison of serum concentration profiles following intravenous (Figure 5.1) and oral (Figure 5.3) administration revealed the presence of both CYC and NCYC in those samples taken following the oral doses. This indicates that the intravenous mode of administration bypasses some metabolic process and limits the chance of "first pass metabolism" of CYC in either the gut wall or in the liver. Pond and Tozer (198) have reported that it is not uncommon for metabolites to be detected in plasma following oral doses and not intravenous doses as is the case with CYC. Analysis of urine samples again revealed the presence of both CYC and NCYC. A summary of absorption and disposition pharmacokinetic parameters estimated from serum concentration data for phase 1 of the study is presented in Tables 5.4 and 5.5.

Table 5.4 Absorption Data for Cyclizine for Subjects 1 to 6 Following Oral Administration of a Single 50mg Dose of Cyclizine Hydrochloride.

SUB	C_{max} $\mu g/\ell$	t_{max} hr	AUC^{∞} $\mu g/\ell.hr$	$AUMC^{\infty}$ $\mu g/\ell.hr^2$	MRT hr	*MAT hr	‡C_{max}/AUC^{∞} hr	‡‡k_a hr^{-1}	‡F
1	14.06	2.5	230.31	6049.96	26.27	11.13	6.10	2.24	0.41
2	17.78	2.5	296.68	6579.52	22.18	6.55	5.99	1.31	0.49
3	13.41	2.0	292.06	7060.50	24.17	8.53	4.59	0.94	0.38
4	10.24	3.0	294.64	10667.22	36.20	23.63	3.48	0.96	0.49
5	22.42	1.5	359.68	8063.33	22.42	2.67	6.23	2.14	0.58
6	14.67	3.5	327.75	9234.84	28.18	14.15	4.48	1.63	0.46
Mean ± S.D.	15.43 3.83	2.0 0.82	300.19 39.35	7942.56 1598.24	26.57 4.79	11.11 6.64	5.15 1.03	1.54 0.52	0.47 0.06

* Calculated using equation 5.20
‡ Reported as ($100\ C_{max}/AUC^{\infty}$)
‡‡ Determined by "method of residuals"
‡ Calculated using equation 5.1 and data from phase 1

Serum concentration time profiles (Figure 5.3) show short lag times of between 15 and 30 minutes for several volunteers. Cyclizine (pK_a = 8.2) is a weakly basic drug that would be highly ionized in the acidic environs of the gastric contents, with the result that insignificant absorption of the drug would occur from the stomach. The pH-partition theory states that the non-ionized form of a drug in solution would be absorbed by diffusion through biological membranes (199). Absorption of CYC from the stomach in the ionized form is unlikely to have occurred despite evidence that absorption of ionized species through the intestinal mucosa is possible (164). Therefore, absorption of CYC most likely occurred in the small intestine where the drug would exist in a predominantly unionized form. Dissolution rate tests on CYC tablets suggest that rapid dissolution occurs and that dissolution rate limited absorption of the drug would be unlikely. Therefore, observed lag times for absorption after oral administration of CYC could well be a result of delay in gastric emptying.

Absorption rate constants (k_a) were calculated for each subject using the "method of residuals". Absorption rates were fairly rapid with absorption half-lives ranging between 20 minutes and 40 minutes for the fastest and slowest absorbers respectively.

C_{max} and t_{max} values were obtained directly from the individual serum concentration versus time data. The average C_{max} value of 15.43 $\pm$ 3.83 $\mu g/\ell$ is lower than the previously reported levels of 69 $\mu g/\ell$ (46) and 49 $\mu g/\ell$ (47) but the mean t_{max} of 2.0 $\pm$ 0.82 hours is in close agreement with values of between two and three hours previously reported. Whilst pilot studies provide initial and useful pharmacokinetic estimates they are usually performed on a single subject and thus results may be tenuous. In addition, drugs that are susceptible to first-pass elimination are known to show pronounced interindividual variations in plasma concentrations (198). Differences in C_{max} values from different studies are therefore most likely to be a result of subject variability. C_{max} and t_{max} are considered crude estimates of absorption rate for bioequivalence studies. Consequently, the ratio of C_{max} and AUC^{∞} was calculated as a more precise and unambiguous estimate of absorption rate as it is independent of the extent of absorption (182).

CYC was found to be approximately 50 percent bioavailable when administered orally and is thus more than likely susceptible to first pass metabolism in the gut wall, a site known to reduce bioavailability of a drug (200) and/or the liver. The absolute bioavailability (F) of orally administered CYC compared to an intravenous reference dose was calculated using equation 5.1. An accurate value for F is essential for the determination of V_Z (equation 5.15) and Cl_{TOT} (equation 5.12) following the oral administration of a compound. Estimation of Cl_{TOT} and V_Z in the absence of F precludes calculation of a true value for the parameter. Rather, the determined numerical value is a ratio of Cl_{TOT} and F or V_Z and F.

Table 5.5 Disposition Data for Cyclizine for Subjects 1 to 6 Following Oral Administration of a Single 50mg Dose of Cyclizine Hydrochloride.

SUB	λ_Z hr^{-1}	$t_{½}$ hr	Cl_{TOT} ℓ/hr/kg	Cl_R ℓ/hr/kg	$^{*}Cl_{NR}$ ℓ/hr/kg	$^{**}V_{ss}$ ℓ/kg	V_Z ℓ/kg	**NCYC %	*CYC %
1	0.0354	19.58	1.030	0.00486	1.025	26.61	29.10	1.22	0.15
2	0.0451	15.37	0.733	0.00982	0.723	15.71	16.27	1.85	0.61
3	0.0441	15.71	0.784	0.00585	0.778	18.12	17.78	1.16	0.35
4	0.0289	23.98	0.881	0.00435	0.877	30.99	31.25	0.99	0.34
5	0.0461	15.03	0.877	0.00649	0.871	19.22	18.99	0.82	0.48
6	0.0378	18.33	0.908	0.00957	0.898	24.42	24.01	1.04	0.39
Mean	0.0396	18.00	0.869	0.00682	0.862	22.51	22.90	1.18	0.39
± S.D.	0.0062	3.15	0.094	0.00214	0.095	5.30	5.69	0.33	0.14

* $Cl_{NR} = Cl_{TOT} - Cl_R$

** Percent of dose of norcyclizine excreted in urine corrected for cyclizine

* Percent of dose of cyclizine excreted in urine

** V_{ss} calculated using equation 5.18

The elimination rate and terminal half-life were calculated as described in § 5.2.5.1. The terminal elimination half-life of 18.00 ± 3.15 hours following oral administration of CYC is in closer agreement with those previously reported (46,47).

The CL_{TOT} of a drug should remain constant irrespective of the route of administration if it follows linear kinetics and no diseased state prevails in the subject. Collier and Riegelman have stated that a change in λ_Z between successive doses of the same drug may be due to changes in either clearance, V_Z, or both (201). The apparent decrease in elimination rate of CYC following oral dosing would be manifested as a decrease in CL_{TOT} if saturation of elimination mechanisms had occurred. The average Cl_{TOT} following a single oral dose of CYC (50mg) was 0.869 ± 0.094 ℓ/hr/kg which is similar to the average Cl_{TOT} of 0.865 ℓ/hr/kg ± 0.096 obtained following an intravenous dose of CYC. The V_Z of the drug following the single oral dose was higher than that calculated following the intravenous dose corresponding to the apparently slower elimination rate, thus maintaining a constant Cl_{TOT} because of the proportional relationship of λ_Z and V_Z to CL_{TOT}. To accurately determine Cl_{TOT} and V_Z the unique value for F obtained for each volunteer was used to calculate the CL_{TOT}, V_{ss} and V_Z for that individual. The average renal clearance for CYC was 0.00682 ± 0.00214 ℓ/hr/kg, which is similar to that following intravenous administration, further emphasising the fact that CYC is primarily eliminated from the body via non-renal pathways.

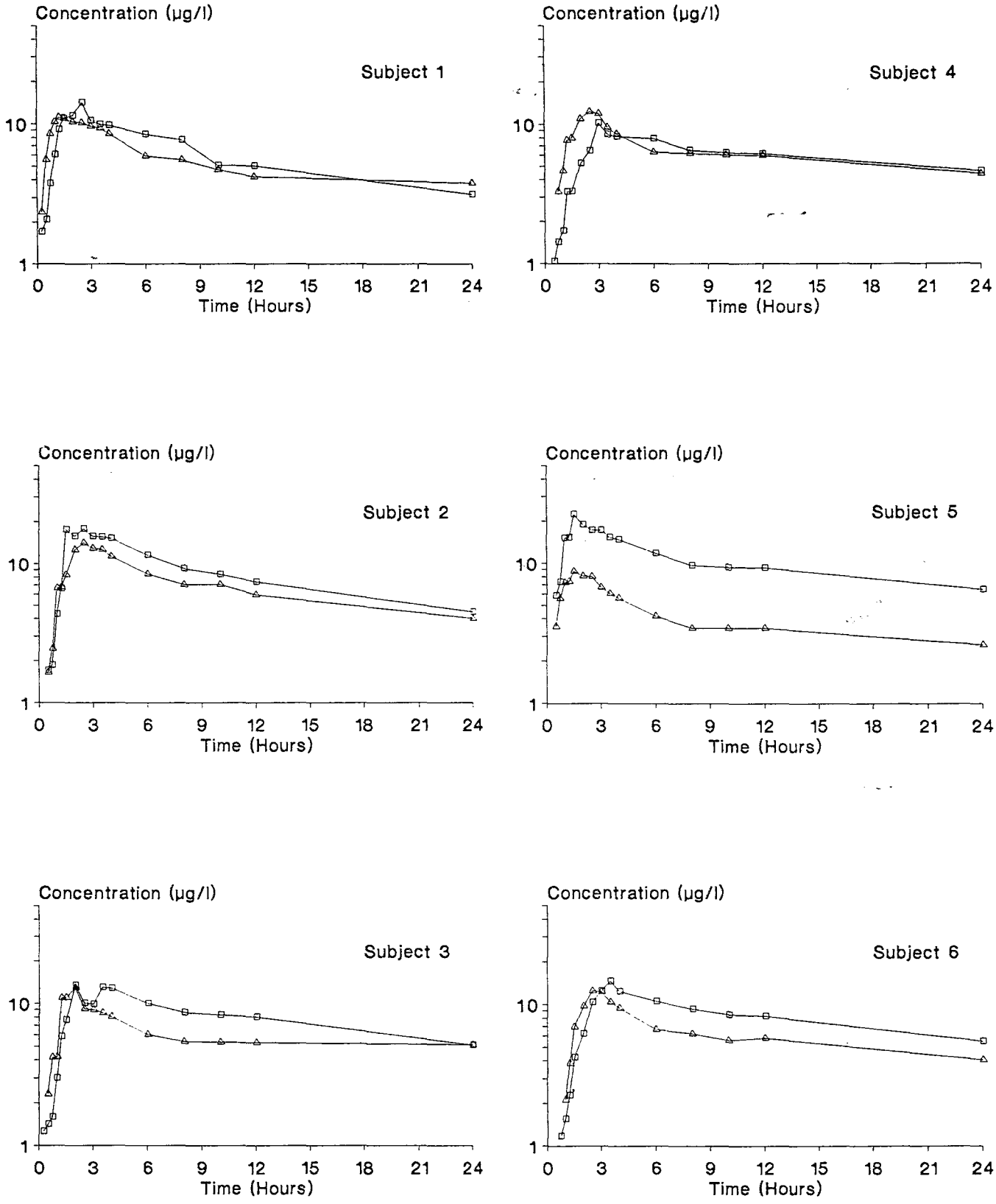


Figure 5.3 Semilogarithmic serum concentration time profiles for subjects 1 to 6 showing both norcyclizine (▵) and cyclizine (□) following administration of a single oral dose (50mg) of cyclizine hydrochloride.

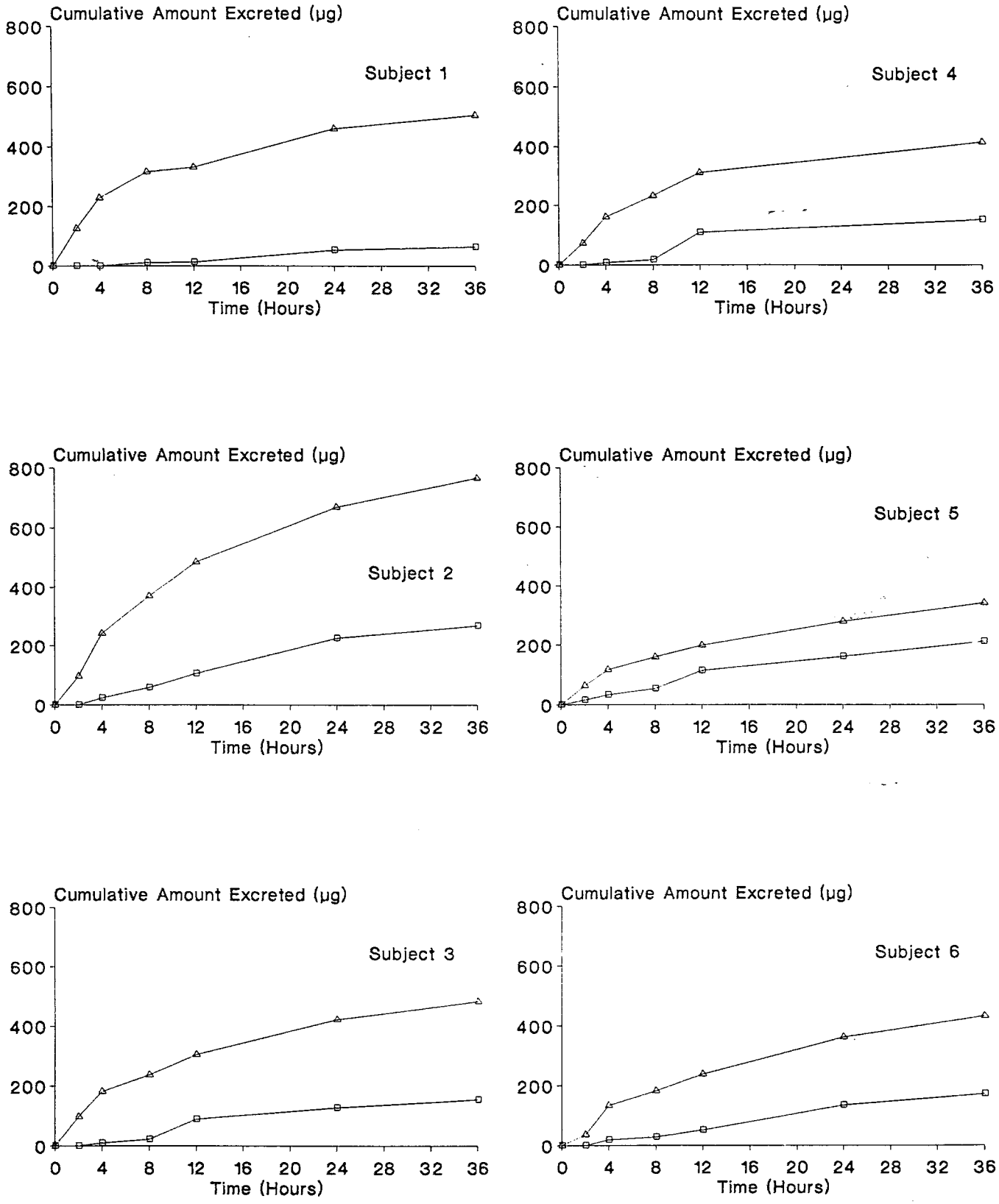


Figure 5.4 Cumulative urinary excretion profiles for subjects 1 to 6 for both norcyclizine (△) and cyclizine (□) following administration of a single oral dose (50mg) of cyclizine hydrochloride.

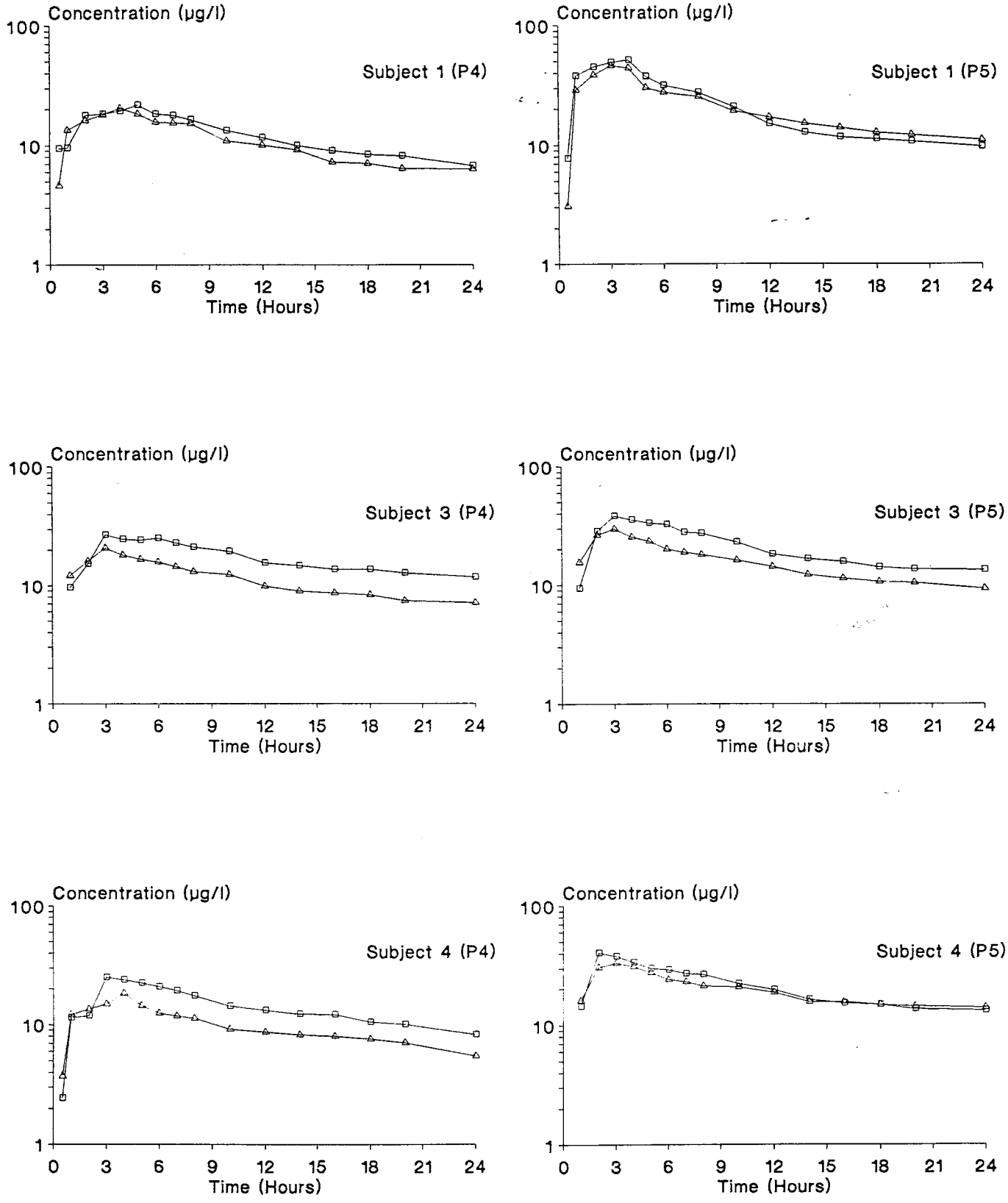


Figure 5.5 Semilogarithmic serum concentration time profiles for subjects 1, 3 and 4 showing both norcyclizine (△) and cyclizine (□) following administration of single oral doses of 100mg (P4) and 150mg (P5) of cyclizine hydrochloride.

5.3.2.2 Phases 4 and 5

In an attempt to determine whether cyclizine exhibits non-linear pharmacokinetics, a dose proportionality study was performed. The absorption and disposition pharmacokinetic parameters obtained following administration of 100mg and 150mg as a single dose to each of three volunteers are listed in Tables 5.6 and 5.7. Semilogarithmic plots of serum concentration versus time for both cyclizine and norcyclizine for these studies are depicted in Figure 5.5.

Table 5.6 Absorption Data for Cyclizine for Subjects 1,3 and 4 Following Oral Administration of Single 100mg and 150mg Doses of Cyclizine Hydrochloride.

DOSE & SUB	C_{max} $\mu g/\ell$	t_{max} hr	AUC^{∞} $\mu g/\ell.hr$	$AUMC^{\infty}$ $\mu g/\ell.hr^2$	MRT hr	*MAT hr	"C_{max}/AUC^{∞} hr	""k_a hr^{-1}	F
100mg S1	21.78	5.00	471.20	11942.36	25.34	10.18	4.62	1.06	0.42
100mg S3	26.69	3.00	861.15	34307.92	39.84	24.19	3.10	1.46	0.56
100mg S4	24.98	3.00	523.59	12645.90	24.15	11.58	4.77	1.34	0.43
Mean ± S.D.	24.48 2.04	3.66 0.94	618.65 172.80	19632.06 10381.37	29.78 7.13	15.32 6.30	4.16 0.75	1.29 0.17	0.47 0.06
150mg S1	51.51	4.00	898.35	28953.67	32.23	17.07	5.73	1.57	0.54
150mg S3	38.09	3.00	963.41	34501.43	35.81	20.17	3.95	0.91	0.42
150mg S4	40.37	2.00	903.72	28511.94	31.55	18.98	4.47	2.18	0.49
Mean ± S.D.	43.32 5.86	3.00 0.82	921.83 29.48	30655.68 2725.33	33.19 1.87	18.74 1.28	4.72 0.75	1.55 0.52	0.48 0.05

* Calculated using equation 5.20
" Reported as (100 C_{max}/AUC^{∞})
"" Determined by "method of residuals"

Table 5.7 Disposition Data for Cyclizine for Subjects 1,3 and 4 Following Oral Administration of Single 100mg and 150mg Doses of Cyclizine Hydrochloride.

DOSE & SUB	λ_Z hr^{-1}	$t_{½}$ hr	"Cl_{TOT} ℓ/hr/kg	""V_{ss} ℓ/kg	"""V_Z ℓ/kg
100mg S1	0.0381	18.19	1.032	25.18	27.08
100mg S3	0.0249	27.83	0.784	30.96	31.47
100mg S4	0.0428	16.19	0.870	20.37	20.34
Mean ± S.D.	0.0353 0.0075	20.74 5.08	0.895 0.103	25.50 4.33	26.30 4.58
150mg S1	0.0253	27.39	1.044	32.98	41.25
150mg S3	0.0265	26.15	0.788	27.36	29.73
150mg S4	0.0303	22.87	0.844	26.26	27.87
Mean ± S.D	0.0274 0.0021	25.47 1.91	0.892 0.109	28.86 2.94	32.95 5.92

" Cl_{TOT} calculated using equation 5.12
"" V_{ss} calculated using equation 5.17
""" V_Z calculated using equation 5.15

It is evident that following single doses of 50mg, 100mg and 150mg of cyclizine hydrochloride that there are few differences in the absorption and disposition kinetics of the drug. The absorption rate and absolute bioavailability factor (F) are similar, and the Cl_{TOT} of the drug is the same following all three dose sizes despite apparent differences in the elimination half-life of the drug calculated using the λ_z value for individual subjects. The reliability of data used in the calculation of the terminal elimination half-life of a drug is dependent on the accuracy with which the last few measured concentrations are determined. It is evident, that despite collection of additional samples between the 12 hours and 24 hours after dosing in order to characterise the elimination of cyclizine completely in phases 4, and 5, no large differences were found in the terminal elimination rate for cyclizine when compared to that calculated from data collected in phase 2, emphasising the reliability with which low concentrations of cyclizine can be determined and the data used with confidence.

A change in the systemic clearance of a drug would be evidence of possible dose-dependent kinetics as a result of saturation of enzyme systems or elimination pathways. Further, comparisons of C_{max} and AUC^{∞} values obtained in phases 1, 4 and 5, by plotting C_{max} and AUC^{∞} values against dose size and assessment of linearity of these plots would provide supplementary proof as to whether the drug behaves non-linearly on administration of higher doses. Plots of mean C_{max} and AUC^{∞} versus dose size of cyclizine hydrochloride are shown in Figures 5.6 and 5.7. Results of two sample *t*-tests for the null hypothesis that non-linearity exists are summarised in Table 5.8. These results show that at the 95% confidence interval the hypothesis can be rejected in all cases. However, the sample size for these tests is small and therefore the power of the statistical test may be in question. Nevertheless, it is apparent that CYC does not show a tendency to follow non-linear kinetics even at dosages that exceed the normal dose range and hence would not be expected to exhibit non-linearity in normal doses of 12.5mg, 25mg and 75mg. Small doses such as 12.5mg and 25mg administered orally would result in extremely low concentrations of cyclizine which could not be measured using the current HPLC method.

Table 5.8 Results of Two-Sample *t*-Test For C_{max} and AUC^{∞} Following Administration of 50mg, 100mg and 150mg Cyclizine Hydrochloride to Human Volunteers.

C_{max} $\mu g/\ell$	50mg	100mg	150mg
50mg		***0.238*** (0.824)	***-1.030*** (0.361)
100mg	***0.238*** (0.824)		***-1.412*** (0.231)
150mg	***-1.030*** (0.361)	***-1.412*** (0.231)	
AUC^{∞} $\mu g/\ell$.hr			
50mg		***-0.651*** (0.550)	***-0.645*** (0.554)
100mg	***-0.651*** (0.550)		***0.261*** (0.807)
150mg	***-0.645*** (0.554)	***0.261*** (0.807)	

NB. Bold Figures represent computed *t*-statistic, and Figures in parentheses the *t*-statistic for comparison.

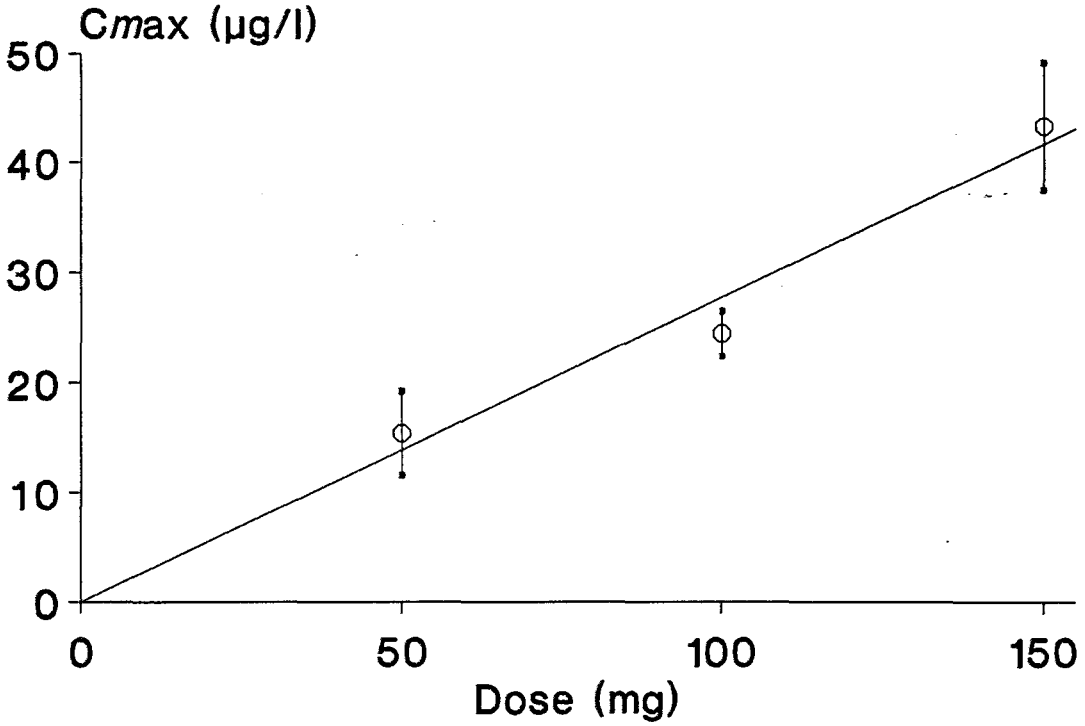


Figure 5.6 Plot of C_{max} versus dose to illustrate dose linearity. Linear regression equation:- y= 0.2789x -0.1467. Correlation coefficient (R^2) = 0.9605.

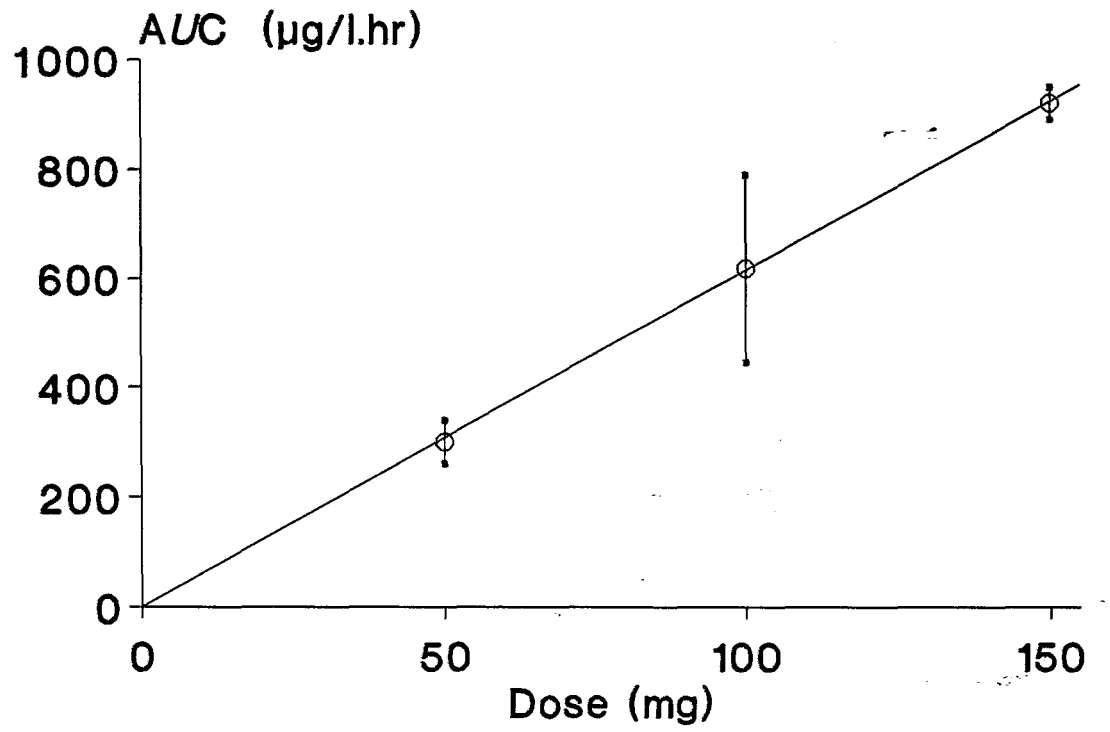


Figure 5.7 Plot of AUC^{∞} versus dose to illustrate dose linearity. Linear regression equation:- y= 6.216x - 8.083. Correlation coefficient (R^2) = 0.9997.

5.3.3 Multiple Dose Study (Phase 3)

Serum samples collected after the last dose following administration of one 50mg cyclizine hydrochloride tablet every 8 hours for five days to 6 subjects were extracted and analyzed by HPLC (§ 3.1.7). The serum concentration time profiles for both cyclizine and norcyclizine are depicted in Figure 5.8 and a summary of pharmacokinetic parameters calculated from these multiple dose data is recorded in Table 5.9.

Table 5.9 Multiple Dose Pharmacokinetic Data for Cyclizine for Subjects 1 to 6 Following Oral Administration of 50mg Cyclizine Hydrochloride Every 8 Hours for Five Days.

SUB	1	2	3	4	5	6	Mean ± S.D.
$AUC^{\tau n \rightarrow \tau n+1}$ $\mu g/\ell.hr$	327.71	405.56	528.09	351.51	608.24	412.68	438.97 ± 98.68
λ_z hr^{-1}	0.0331	0.0317	0.0374	0.0225	0.0305	0.0339	0.0315 ± 0.005
$t_{½}$ hr	20.94	21.86	18.52	30.80	22.72	20.44	22.55 ± 3.91
Cl_{TOT} ℓ/hr/kg	0.813	0.537	0.513	0.709	0.518	0.721	0.635 ± 0.117
V_z ℓ/kg	24.55	16.95	13.73	31.49	16.98	21.27	20.82 ± 5.89
¶C^{ss}_{av} $\mu g/\ell$	40.96	50.70	66.01	43.94	76.03	51.59	54.87 ± 12.35
¶C^{ss}_{max} $\mu g/\ell$	46.62	57.36	76.39	48.02	85.68	58.59	62.11 ± 14.33
¶C^{ss}_{min} $\mu g/\ell$	35.78	44.51	56.63	40.11	67.13	44.90	48.17 ± 10.60
♦C^{ss}_{av} $\mu g/\ell$	28.79	37.09	36.51	36.83	44.96	40.97	37.53 ± 4.92
■C^{ss}_{max} $\mu g/\ell$	51.92	59.28	87.16	59.07	86.44	59.60	67.25 ± 14.07
■C^{ss}_{min} $\mu g/\ell$	36.14	41.30	54.71	37.76	64.45	49.46	47.30 ± 10.04
R (τ=8)	4.06	3.30	3.36	4.85	3.24	3.83	3.77 ± 0.57
R (τ=24)	1.75	1.51	1.53	1.99	1.49	1.68	1.66 ± 0.18
*PTF %	26.46	25.35	29.93	18.00	24.39	26.54	25.11 ± 3.61

¶ Values calculated using pharmacokinetic parameters calculated after a single dose
♦ C^{ss}_{av} estimated using AUC^{∞} following a single oral dose of 50mg
■ Values read directly from blood level data
* PTF = Peak-trough fluctuations

Area under the curve for one dose interval ($AUC^{\tau n \rightarrow \tau n+1}$) was calculated as described in § 5.2.3.2 and these data used to calculated the average steady state concentration for each subject using equation 5.22. If a drug exhibits linear pharmacokinetics on increased dosing, then information obtained following a single oral dose of that drug would enable prediction of average steady state concentrations. It is well established that AUC^{∞} after a single dose is equivalent to $AUC^{\tau n \rightarrow \tau n+1}$ after multiple dosing to steady state and therefore the quotient of AUC^{∞} following a single dose and the dosing interval (τ) is the expected C^{ss}_{av}. However, average steady state levels predicted following a single oral dose of 50mg of cyclizine hydrochloride were lower than those levels actually obtained following multiple oral doses of the drug. The mean C^{ss}_{av} predicted following a single oral dose to each of six volunteers was 37.53 ± 4.92 $\mu g/\ell$ as opposed to the value of 54.87 ± 12.35 $\mu g/\ell$ obtained using $AUC^{\tau n \rightarrow \tau n+1}$ following dosing every 8 hours for five days, suggesting that the drug accumulates in a non-linear fashion. In addition, the ratio of AUC^{∞} and $AUC^{\tau n \rightarrow \tau n+1}$ would provide information with respect to enzyme induction or inhibition (68). The ratio of mean AUC^{∞} (phase 1) and mean $AUC^{\tau n \rightarrow \tau n+1}$ (phase 3) for CYC is 0.684 suggesting enzyme saturation occurs and thus

steady state levels would be higher than predicted. However, data from the dose-dependency study (Phases 4 and 5, § 5.3.2.2) clearly indicates that this does not appear to be the case. An indication of drug accumulation can be obtained using equation 5.26.

$$R = \frac{1}{1-e^{-\lambda_z \tau}} \qquad \text{Eq. 5.26}$$

This measure of drug accumulation or the accumulation index (R) calculated using equation 5.26 is independent of the dose and is highly dependent on the dose interval (τ) and elimination constant (λ_Z). The average value for R of 3.77 $\pm$ 0.57 calculated using a dose interval of 8 hours indicates that substantial accumulation would occur as opposed to if a dose interval of 24 hours was used, which explains why cyclizine accumulated to a higher level than originally predicted. Even though 8 hourly dosage intervals are recommended (41) the interval is much shorter than the $t_{½}$ for the drug and therefore such a dosing interval is inappropriate as has clearly been shown in the foregoing discussions.

C^{ss}_{av} was estimated using $AUC^{\tau \rightarrow \tau n+1}$ as the value of AUC^{∞} following the single 50mg dose of cyclizine hydrochloride underpredicted C^{ss}_{av}. The relationship between AUC^{∞} and $AUC^{\tau n \rightarrow \tau n+1}$, if the drug follows linear kinetics, allows Cl_{TOT} to be calculated using equation 5.12. The terminal rate constant was calculated by linear regression of the terminal slope of a semilog plot of serum concentration data versus time. Cl_{TOT} was estimated using equation 5.12 and the volume of distribution calculated from these data. Values for C^{ss}_{max} and C^{ss}_{min} were computed with equations 5.24 and 5.25 respectively and these values were used to compute the bioequivalence parameter, percent peak-trough fluctuation. The computed values of 62.11 $\pm$ 14.33 $\mu g/\ell$ and 48.17 $\pm$ 10.60 $\mu g/\ell$ for C^{ss}_{max} and C^{ss}_{min} respectively are similar to the values of 67.25 $\pm$ 14.07 $\mu g/\ell$ (C^{ss}_{max}) and 47.30 $\pm$ 10.04 $\mu g/\ell$ (C^{ss}_{min}) observed directly from the blood level data. This provides confirmation that the pharmacokinetic parameters determined following a single dose of cyclizine characterizes the disposition of the drug correctly. The absolute bioavailability factor (F), when required for these calculations, was an average value of those calculated for each subject in the single dose studies.

The average elimination half-life of CYC was 22.55 $\pm$ 3.91 hours and was in close agreement with the half-life calculated following administration of single 50mg, 100mg and 150mg doses of cyclizine hydrochloride. The Cl_{TOT} of 0.635 ℓ/hr/kg was slower than the 0.8 ℓ/hr/kg following the intravenous and single oral doses of cyclizine lactate and cyclizine hydrochloride. The predicted C^{ss}_{av} concentration following the initial 50mg single dose study was approximately 23 percent lower than

the computed C^{ss}_{min} (48.17 $\pm$ 10.60 $\mu g/\ell$) and 40 percent lower than the computed C^{ss}_{max} (62.11 $\pm$ 14.33 $\mu g/\ell$). The percent peak-trough fluctuation was 25.11 $\pm$ 3.61.

A major limitation of multiple dose studies is that the assumptions for calculating parameters are unfounded if the subject is not at steady state. However, visual assessment of profiles obtained following multiple dosing shows that the subjects were at steady state. In addition, it is known to take approximately 3.32 half-lives to reach 90 percent of steady state levels. Therefore, to reach this level cyclizine with a half-life of approximately 20 hours would have to be dosed for 66.4 hours. In this study cyclizine was dosed for 120 hours to ensure that subjects were at steady state.

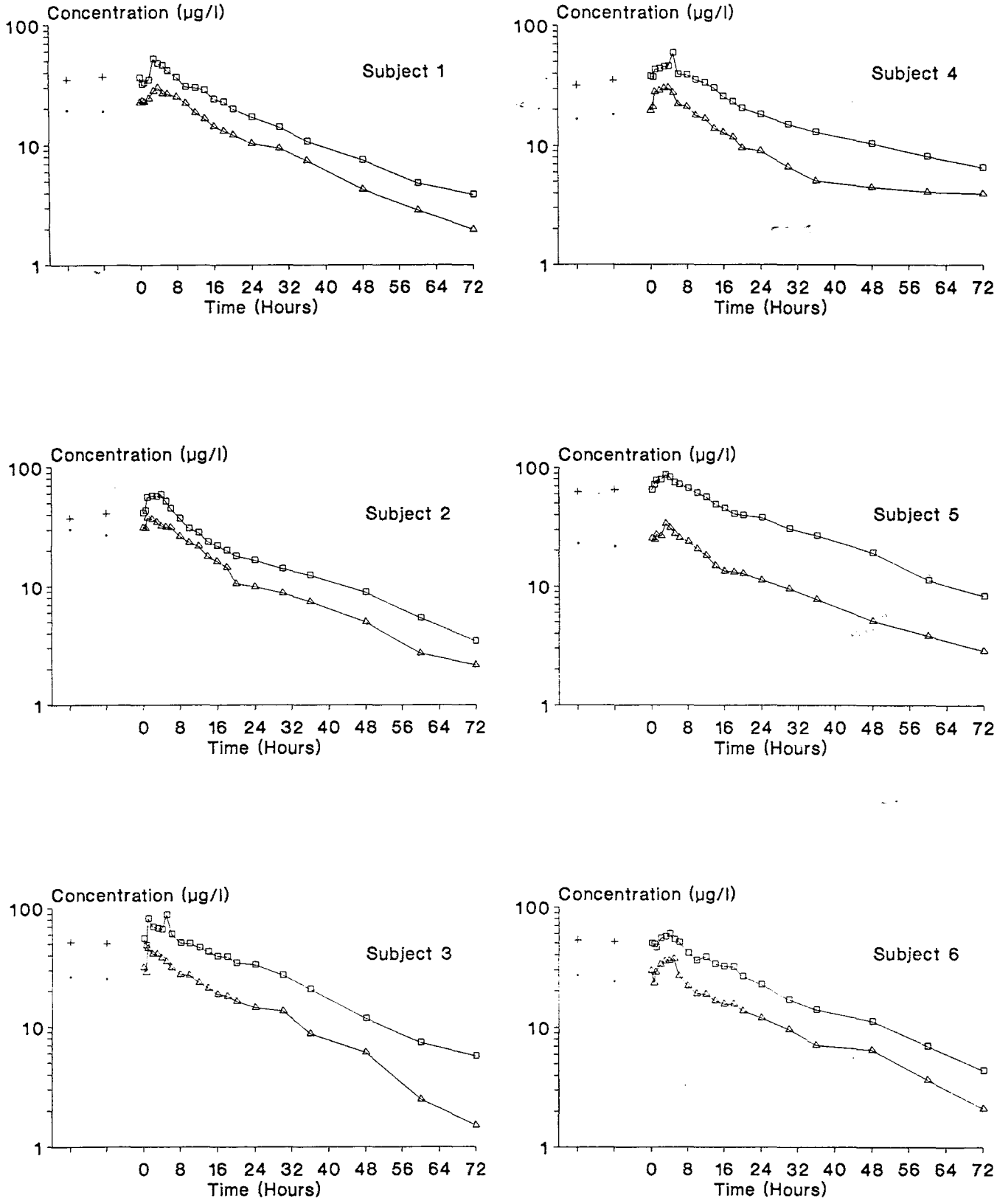


Figure 5.8 Semilogarithmic serum concentration time profiles for subjects 1 to 6 showing norcyclizine (▵) and cyclizine (□) following multiple dosing with 50mg of cyclizine hydrochloride every eight hours for 5 days showing trough concentrations for norcyclizine (■) and cyclizine (+) before the 14th and 15th doses.

5.3.4 Compartmental Analysis

All single dose and multiple dose data were fitted with the aid of a nonlinear curve fitting programme PCNONLIN (SCI Software, Lexington, KY, U.S.A.) to models selected from the PCNONLIN library (202) (see Appendix 1). Goodness of fit was determined by sum of squared residuals (SSR) in conjunction with the correlation coefficient (R^2). The model that best described the observed serum concentration versus time data was selected using Akaike's information criterion (AIC) (203). AIC was defined by Akaike (204) and Tanabe (205) based on the assumption that random errors obey Gaussian distribution and is calculated using equation 5.26.

$$AIC = N \ln R_e + 2p \qquad \text{Eq. 5.27}$$

where N = number of experimental data points,
R_e = residual sum of squares, and,
p = number of parameters in the estimated model.

The lowest value determined for AIC for a particular equation is regarded as the one that best represents the observed data for the serum concentration versus time plots.

5.3.4.1 Intravenous Dosing (Phase 1)

Serum concentrations obtained following IV administration of cyclizine were fitted to PCNONLIN Models 1, 7 and 18. The results of fitting observed data to PCNONLIN Model 1 which describes a 1BCM model following an IV bolus dose with first order elimination or output are summarized in Table 5.10. PCNONLIN Model 7 describes a 2BCM model following an IV bolus dose with first order output using microconstants as parameters (*viz.* k_{12}, k_{21}). The results of fitting IV data to PCNONLIN Model 7 are summarized in Table 5.11. PCNONLIN Model 18 describes the simplest of 3BCM models following intravenous administration, in which elimination occurs from a central compartment which is reversibly connected to a "shallow" and a "deep" peripheral compartment, compartments 2 and 3 respectively (172). The results of fitting data to PCNONLIN Model 18 are summarized in Table 5.12. Plots of experimental and fitted data for 1BCM, 2BCM and 3BCM modelling are depicted in Figures 5.9, 5.10 and 5.11 respectively.

Assessment of the data listed in Tables 5.10, 5.11 and 5.12 and visual appraisal of Figures 5.9, 5.10 and 5.11 clearly illustrate that the disposition of CYC following intravenous administration is better described by the 3BCM, based on AIC, correlation coefficients and sum of squared residuals. Despite a reasonable fit for the 2BCM, parameter estimates were not in close agreement with those

parameters determined by noncompartmental methods. The 2BCM model is unable to account for the slow terminal elimination rate of cyclizine and therefore the determined mean terminal half-life of 4.12 hours is misleading as seen by the half-life of approximately 14 hours obtained from non-compartmental data analysis. The elimination half-life of 13.97 hours obtained from fitting data from subject 6 hours closely approximates the half-life estimated using non-compartmental techniques. In general, values for λ_Z obtained following fitting of data to the 3BCM provide close approximations to those calculated previously (Table 5.2) except for subject 6 in which the disposition of cyclizine is best described by a 2BCM. In addition, the V_{ss} was calculated using equation 5.17 for both 2BCM and 3BCM. The equation was modified as described by Gibaldi and Perrier (172) and provision made for an additional exponential term. Parameters estimated from curve fitting to PCNONLIN Model 18 were used to estimate V_{ss} and the results show that cyclizine is indeed distributed to both "shallow" and "deep" tissue compartments. These data are in close agreement with those reported previously (Table 5.2). It is clearly evident that whilst the 2BCM provides reasonable fits, some of the parameter estimates are unrealistic as a result of an inability to account for the extensive distribution of cyclizine. As a result, the elimination half-life is much faster and the V_{ss} smaller than they are in reality. In addition, the difference between V_{ss} and V_c for the 2BCM and 3BCM modelling is large providing further proof that the drug is extensively distributed and that the volume of the tissue compartment is large. The disposition of cyclizine following an intravenous bolus dose is thus better described by use of a 3BCM. Clearance following fitting of data to 2BCM and 3BCM models was calculated using equation 5.11 with the AUC^{∞} estimates obtained directly from fitting.

Table 5.10 Results of Compartment Modelling to PCNONLIN Model 1 (1BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following an Intravenous Bolus Dose of Cyclizine Lactate Solution.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	139.20	149.36	136.90	141.08	119.99	102.05	131.43 ± 15.81
**R^2	0.871	0.892	0.895	0.926	0.957	0.983	0.920 ± 0.0391
***SSR	1828	3216	1609	2030	628	232	1591 ± 972
λ_Z hr^{-1}	0.785	1.493	0.680	1.321	0.794	0.336	0.902 ± 0.392
V_c ℓ/kg	4.06	1.95	4.18	2.58	4.03	5.12	3.65 ± 1.06
•Cl ℓ/hr/kg	3.19	2.91	2.85	3.41	3.20	1.72	2.88 ± 0.55

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_Z x V_c

Table 5.11 Results of Compartment Modelling to PCNONLIN Model 7 (2BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following an Intravenous Bolus Dose of Cyclizine Lactate Solution.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	61.84	130.37	102.49	98.30	85.42	90.36	94.79 ± 20.54
**R^2	0.999	0.965	0.984	0.992	0.993	0.990	0.987 ± 0.0108
***SSR	19	896	190	151	74	97	238 ± 299
k_{21} hr^{-1}	2.25	2.38	1.15	1.32	0.86	0.14	1.35 ± 0.78
α hr^{-1}	12.15	17.00	3.89	5.96	2.69	0.54	7.04 ± 5.74
λ_z hr^{-1}	0.200	0.362	0.106	0.159	0.139	0.0436	0.168 ± 0.0991
V_c ℓ/kg	1.39	0.56	2.76	1.62	3.16	4.86	2.39 ± 1.40
V_{ss} ℓ/kg	6.93	3.43	8.43	6.56	8.71	14.44	8.08 ± 3.32
•Cl ℓ/hr/kg	1.55	1.48	0.99	1.15	1.38	0.82	1.23 ± 0.26

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl calculated using equation 5.11

Table 5.12 Results of Compartment Modelling to PCNONLIN Model 18 (3BCM) of Cyclizine Concentration Profiles for Subjects 1 to 5 Obtained Following an Intravenous Bolus Dose of Cyclizine Lactate Solution.

SUB	1	2	3	4	5	Mean ± S.D.
*AIC	37.93	129.38	105.91	98.23	83.65	91.02 ± 30.39
**R^2	1.000	0.973	0.984	0.994	0.995	0.989 ± 0.0096
***SSR	4	679	184	120	54	208 ± 243
k_{21} hr^{-1}	2.49	3.41	1.27	1.78	1.48	2.09 ± 0.78
α hr^{-1}	12.93	19.98	4.07	6.76	3.44	9.44 ± 6.25
k_{31} hr^{-1}	0.0913	0.225	0.0389	0.141	0.104	0.120 ± 0.0618
ß hr^{-1}	0.318	0.969	0.161	0.412	0.397	0.451 ± 0.274
λ_z hr^{-1}	0.0394	0.0533	0.0132	0.0423	0.0254	0.0347 ± 0.0139
V_{ss} ℓ/kg	16.68	10.08	26.79	13.36	21.99	17.78 ± 5.98
V_c ℓ/kg	1.34	0.49	2.74	1.56	3.06	1.84 ± 0.94
•Cl ℓ/hr/kg	0.94	0.67	0.48	0.73	0.69	0.70 ± 0.015

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl calculated using equation 5.11

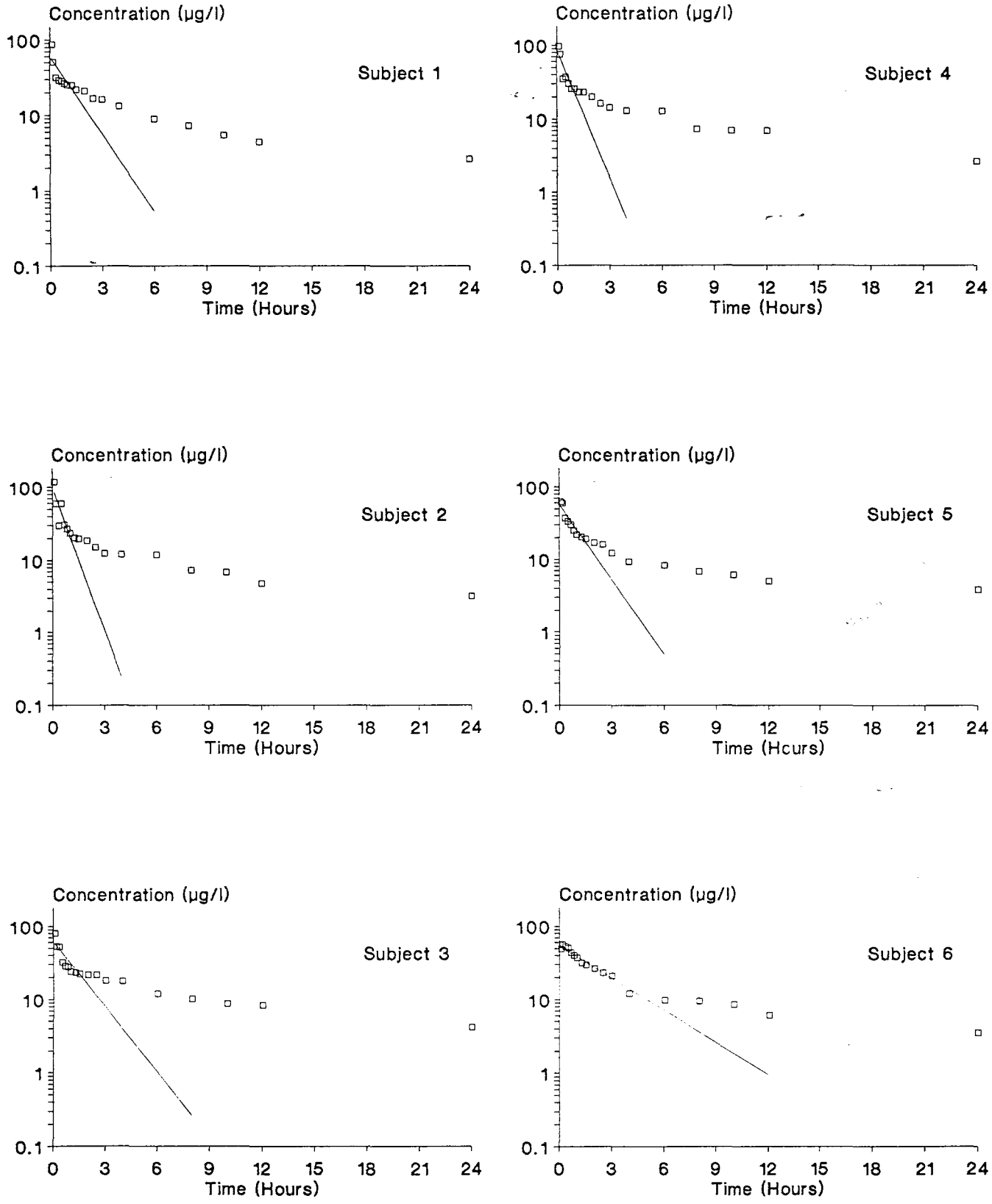


Figure 5.9 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of a single intravenous dose of cyclizine lactate (25mg). Model = PCNONLIN model 1 (1BCM).

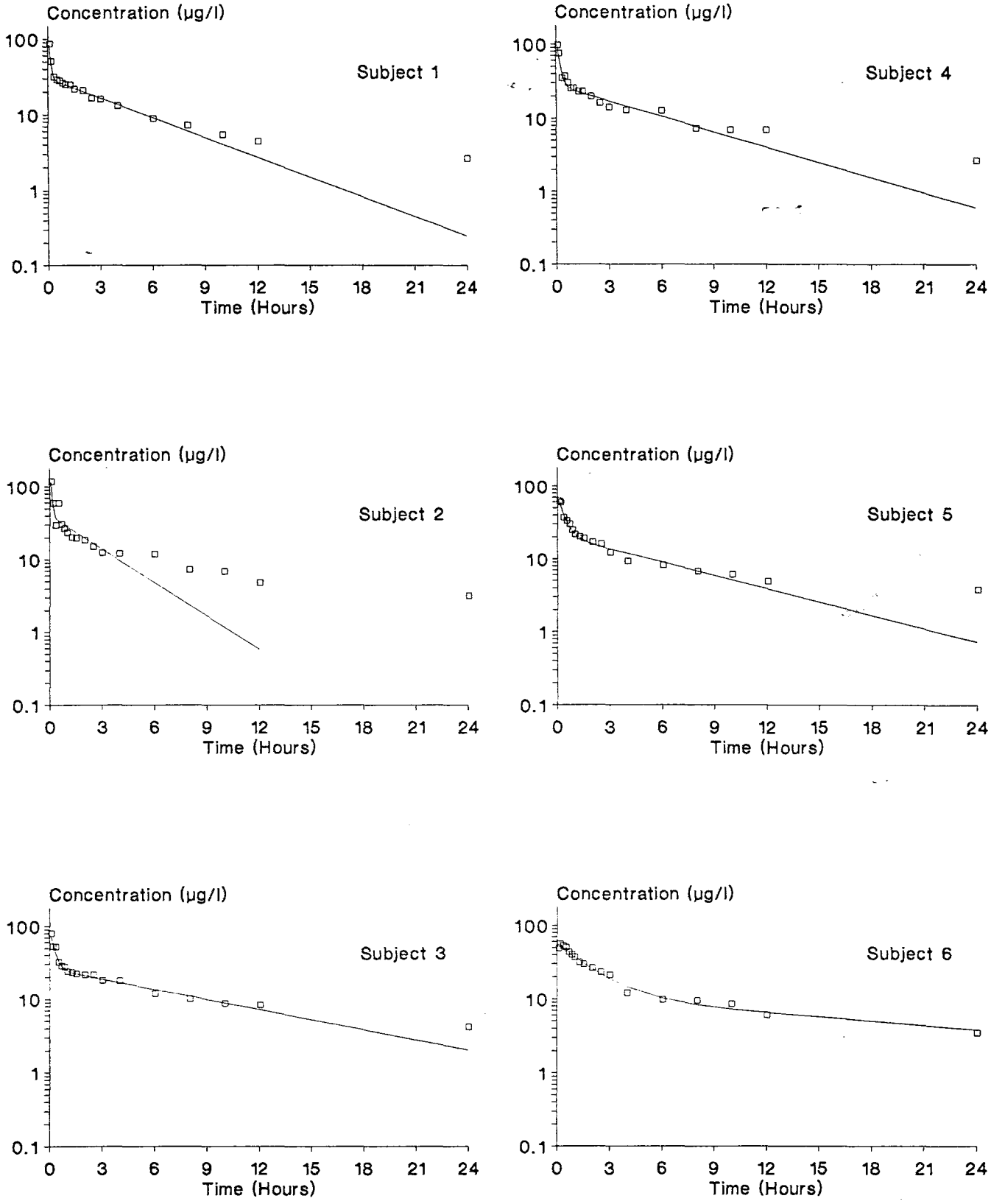


Figure 5.10 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of a single intravenous dose of cyclizine lactate (25mg). Model = PCNONLIN model 7 (2BCM).

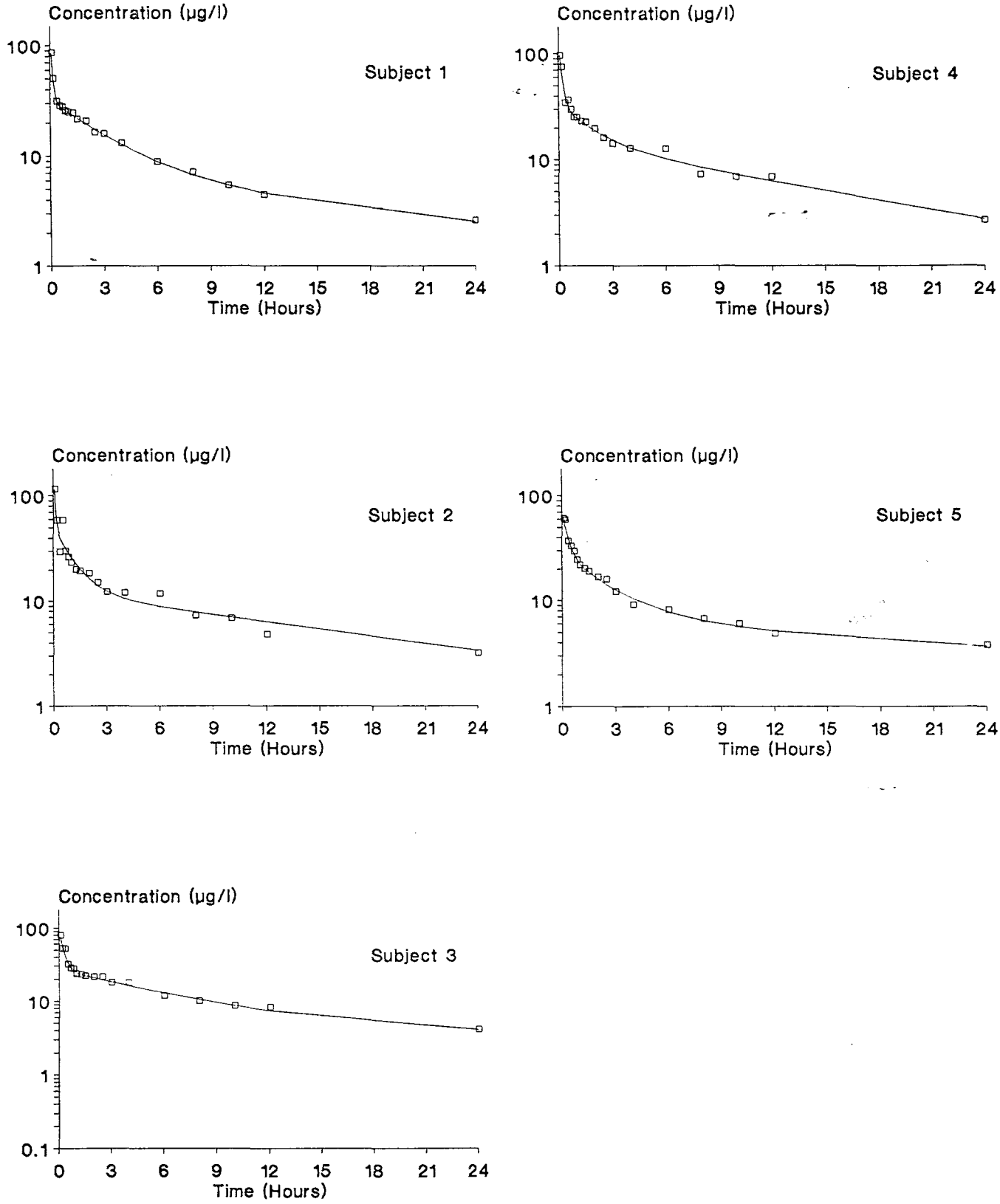


Figure 5.11 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 5 following administration of a single intravenous dose of cyclizine lactate (25mg). Model = PCNONLIN model 18 (3BCM).

5.3.4.2 Oral Dosing (Phase 2)

Serum concentrations obtained following oral administration of cyclizine as a single dose of 50mg were fitted to models 4 and 12. These models, allowing for the provision of a lag time in absorption were selected and applied to all profiles resulting from oral administration of cyclizine. Preliminary fitting of data to PCNONLIN Models 3 (no t_{lag}), 4, 11 (no t_{lag}) and 12 suggested that models 4 and 12 were the most appropriate for describing these profiles. Parameters obtained following fitting of observed data to PCNONLIN Model 4 which describes a 1BCM model following an oral dose with first order absorption and elimination with a lag time are summarized in Table 5.13. PCNONLIN Model 12 defines a 2BCM model with first order absorption and elimination with a lag time following an oral dose using microconstants as parameters (*viz.* k_{12}, k_{21}). The results of fitting oral data to PCNONLIN Model 12 are summarized in Table 5.14. Plots of experimental and fitted data for 1BCM and 2BCM modelling are depicted in Figures 5.12 and 5.13 respectively.

Table 5.13 Results of Compartment Modelling to PCNONLIN Model 4 (1BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following a Single Oral Dose of Cyclizine Hydrochloride.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	59.19	71.97	63.47	52.37	78.39	62.14	64.59 ± 8.47
**R^2	0.962	0.969	0.959	0.957	0.951	0.974	0.962 ± 0.0076
***SSR	20	43	26	14	62	24	32 ± 16
t_{lag} hr	0.43	0.92	0.70	0.68	0.39	1.27	0.73 ± 0.30
k_a hr^{-1}	1.40	2.43	1.25	0.77	2.23	1.21	1.55 ± 0.59
C_{max} $\mu g/\ell$	11.38	16.74	11.63	8.19	17.84	12.32	13.02 ± 3.30
t_{max} hr	2.50	2.32	3.39	4.66	1.95	3.95	3.12 ± 0.96
λ_Z hr^{-1}	0.0939	0.0914	0.0498	0.0419	0.0780	0.0547	0.0683 ± 0.020
V ℓ/kg	41.88	23.35	45.34	57.79	26.95	45.35	40.11 ± 11.72
•Cl ℓ/hr/kg	3.93	2.13	2.26	2.29	2.10	2.48	2.53 ± 0.64

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_Z x V

In general a 2BCM, PCNONLIN Model 12 (Table 5.14), describes the serum concentration time profile of cyclizine following a single oral dose of 50mg of cyclizine hydrochloride better than a 1BCM, PCNONLIN Model 4 (Table 5.13). The absorption rate constant (k_a) obtained following fitting to a 1BCM (Model 4) of 1.55 ± 0.59 hr^{-1} was in close agreement with that determined using the

"method of residuals" (Table 5.4). However, other parameters such as the terminal elimination rate and volume were approximately two fold faster and larger respectively, and total clearance three fold faster than estimated following non-compartmental analysis of data. Parameters such as the terminal elimination rate, volume of distribution and total clearance estimated using the 2BCM, were found to be in close agreement with those determined using noncompartmental techniques. However, the absorption rate constant was much slower than that obtained for the 1BCM and noncompartmental estimation. Both the 1BCM and 2BCM failed to predict values for C_{max} and t_{max} that were similar to those determined directly from the serum concentration time profiles for individual subjects.

Table 5.14 Results of Compartment Modelling to PCNONLIN Model 12 (2BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following a Single Oral Dose of Cyclizine Hydrochloride.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	59.02	73.09	66.30	65.15	72.03	59.83	65.90 ± 5.39
**R^2	0.970	0.973	0.965	0.927	0.973	0.978	0.964 ± 0.0171
***SSR	16	36	24	25	34	17	25 ± 8
t_{lag} hr	0.43	0.90	0.84	0.00	0.37	0.98	0.59 ± 0.35
k_a hr^{-1}	0.60	1.22	0.30	0.39	0.92	0.41	0.64 ± 0.33
C_{max} $\mu g/\ell$	11.78	17.68	11.89	7.95	19.14	12.67	13.52 ± 3.79
t_{max} hr	2.44	2.34	2.98	6.01	1.94	4.24	3.33 ± 1.40
k_{21} hr^{-1}	0.24	0.36	0.23	1.30	0.25	0.11	0.42 ± 0.40
α hr^{-1}	0.77	0.79	1.39	1.30	0.79	0.39	0.91 ± 0.34
λ_z hr^{-1}	0.0431	0.0502	0.0327	0.0498	0.0244	0.0112	0.0352 ± 0.014
V ℓ/kg	19.68	14.41	9.05	49.26	13.79	23.08	21.55 ± 13.17
•Cl ℓ/hr/kg	0.85	0.72	0.29	2.45	0.34	0.26	0.81 ± 0.76

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_z x V

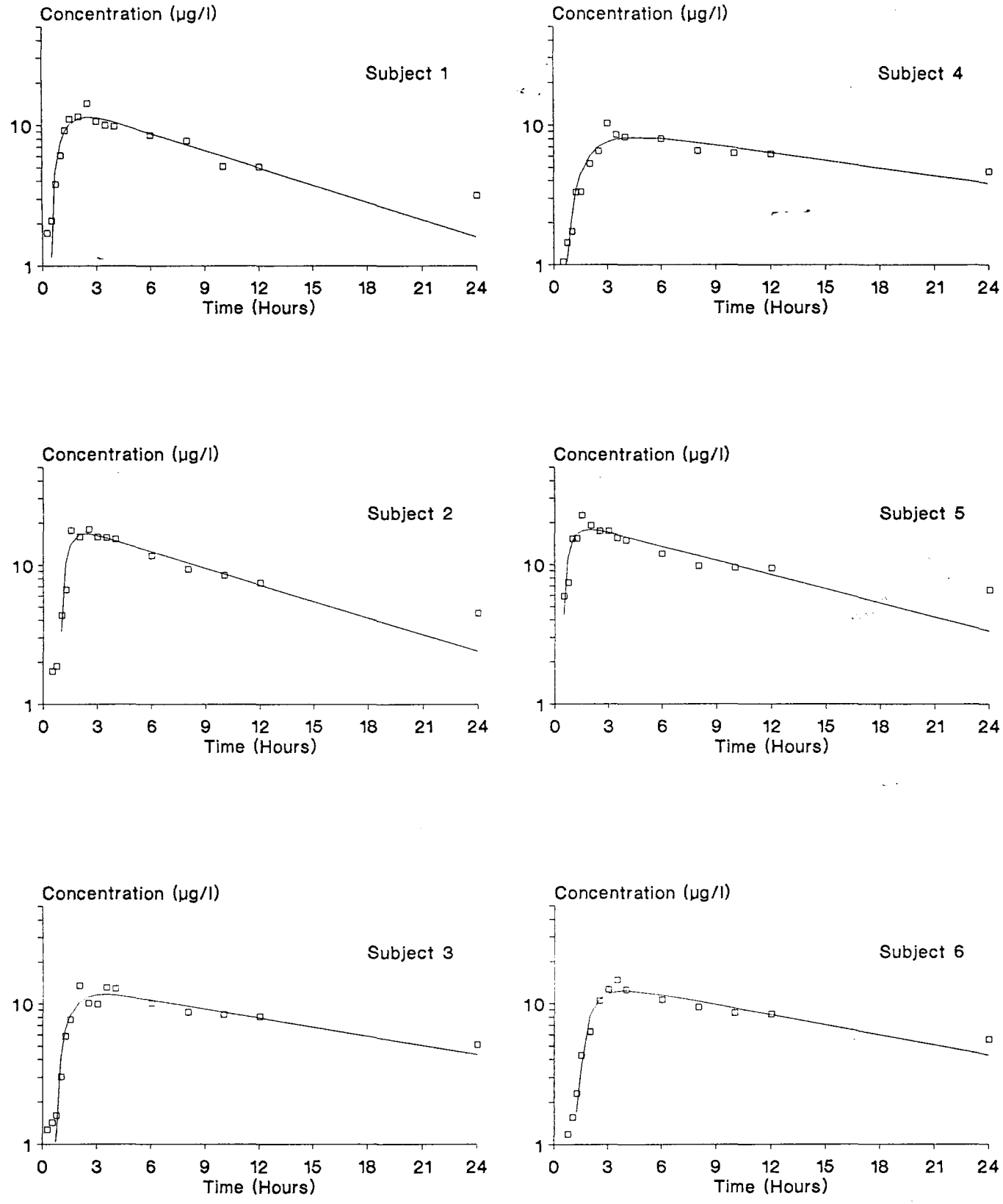


Figure 5.12 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of a single oral dose of cyclizine hydrochloride (50mg). Model = PCNONLIN model 4 (1BCM).

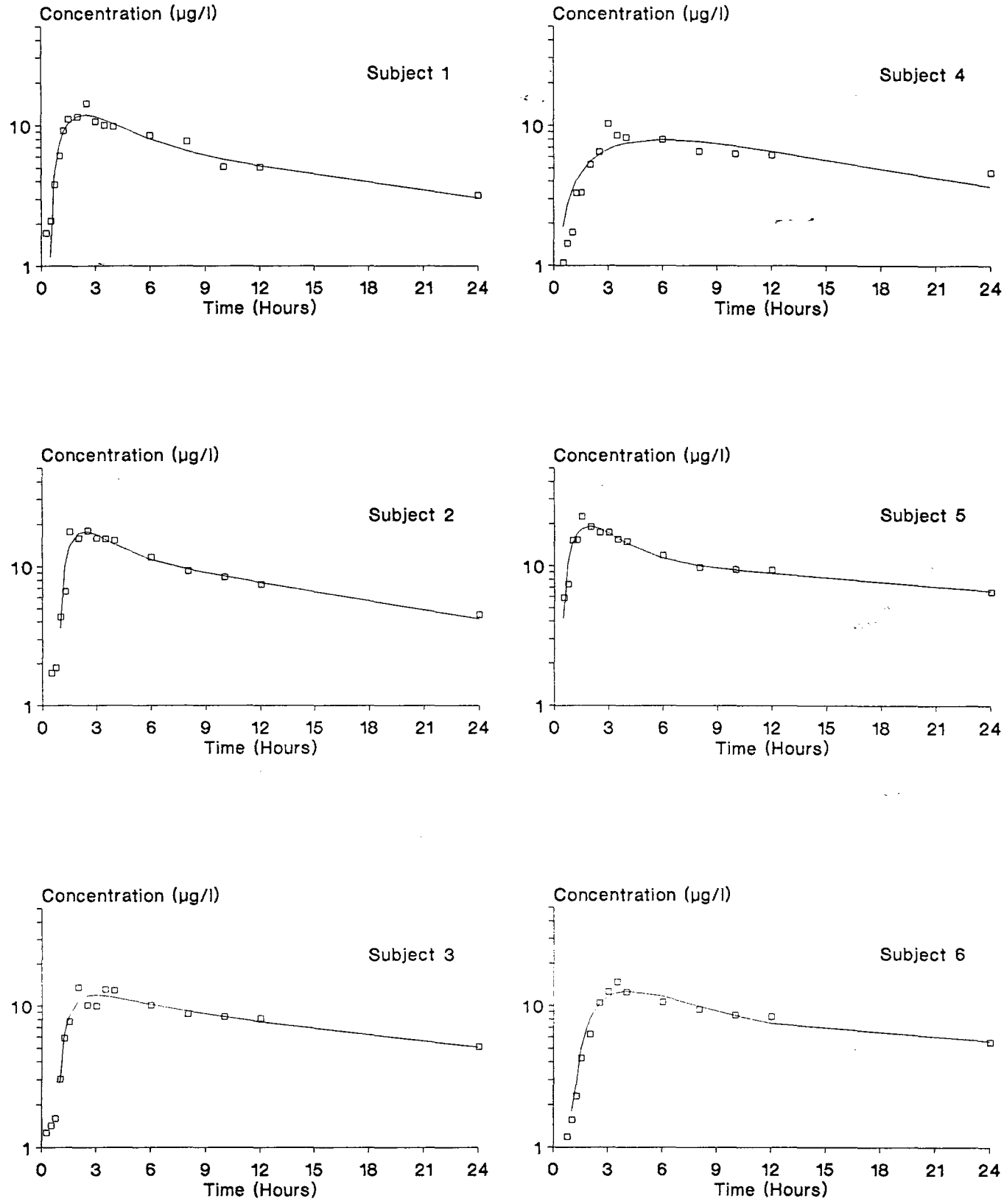


Figure 5.13 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of a single oral dose of cyclizine hydrochloride (50mg). Model = PCNONLIN model 12 (2BCM).

5.3.4.3 Oral Dosing (Phases 4 and 5)

Serum concentrations obtained following oral administration of cyclizine as a single dose of either 100mg or 150mg were fitted to models 4 and 12. Parameters obtained following fitting of observed data to PCNONLIN Model 4 are summarized in Table 5.15 and those obtained following fitting of data to PCNONLIN Model 12 in Table 5.16. Plots of experimental and fitted data for both dose sizes for 1BCM and 2BCM modelling are depicted in Figures 5.14 and 5.15 respectively.

Table 5.15 Results of Compartment Modelling to PCNONLIN Model 4 (1BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following Single Oral Doses of 100mg and 150mg of Cyclizine Hydrochloride.

SUB	1 **100mg**	3 **100mg**	4 **100mg**	Mean ± S.D.	1 **150mg**	3 **150mg**	4 **150mg**	Mean ± S.D.
*AIC	62.57	72.09	80.50	71.72 ± 7.32	89.34	73.54	67.78	76.88 ± 9.11
**R^2	0.977	0.979	0.953	0.969 ± 0.012	0.980	0.989	0.992	0.987 ± 0.0051
***SSR	25	43	71	46 ± 19	161	47	34	81 ± 57
t_{lag} hr	0.00	0.53	0.38	0.30 ± 0.22	0.41	0.79	0.94	0.71 ± 0.22
k_a hr^{-1}	0.64	0.74	0.71	0.69 ± 0.042	1.63	1.18	7.48	3.43 ± 2.87
C_{max} $\mu g/\ell$	19.69	24.55	21.72	21.99 ± 1.99	49.56	35.28	39.17	41.34 ± 6.03
t_{max} hr	3.87	4.41	4.13	4.13 ± 0.22	2.18	3.37	1.58	2.38 ± 0.74
λ_z hr^{-1}	0.0694	0.0516	0.0617	0.0609 ± 0.007	0.109	0.0673	0.0634	0.0799 ± 0.021
V ℓ/kg	44.93	40.19	38.68	41.27 ± 2.66	28.85	43.06	38.97	36.96 ± 5.97
•Cl ℓ/hr/kg	3.12	2.07	2.39	2.53 ± 0.44	3.14	2.89	2.47	2.83 ± 0.28

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_z x V

Goodness of fit criteria, suggest that the disposition of cyclizine is best described by a 2BCM for both the 100mg and 150mg doses. Parameter estimates determined following fitting to a 2BCM were in close agreement to noncompartmental estimates in both cases (Tables 5.6 and 5.7). The estimates obtained following fitting to the 1BCM model showed deviations of a similar magnitude to those observed when data from a 50mg dose were fitted to the 1BCM. Estimates for parameters such as

the terminal elimination rate of 0.0331 ± 0.020 hr^{-1} or 0.0172 ± 0.006 hr^{-1} for the 100mg and 150mg doses respectively are similar to those of 0.0353 ± 0.0075 hr^{-1} or 0.0274 ± 0.0021 hr^{-1} for the same doses, determined using noncompartmental techniques. In addition, parameters such as volume and total clearance were similar to those derived from noncompartmental techniques. However, the estimation of absorption rate was not as precise, with only the fitted value of 1.68 ± 1.34 hr^{-1} determined for the 150mg dose showing similarity to the value of 1.55 ± 0.52 hr^{-1} determined using the "method of residuals".

Table 5.16 Results of Compartment Modelling to PCNONLIN Model 12 (2BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following Single Oral Doses of 100mg and 150mg of Cyclizine Hydrochloride.

SUB	1 100mg	3 100mg	4 100mg	Mean ± S.D.	1 150mg	3 150mg	4 150mg	Mean ± S.D.
*AIC	62.67	68.37	84.61	71.88 ± 9.30	87.21	55.85	49.52	64.19 ± 16.48
**R^2	0.982	0.986	0.953	0.974 ± 0.015	0.986	0.997	0.998	0.994 ± 0.005
***SSR	20	28	72	40 ± 23	110	13	9	44 ± 47
t_{lag} hr	0.00	0.49	0.38	0.29 ± 0.21	0.37	0.74	0.89	0.67 ± 0.22
k_a hr^{-1}	0.44	0.43	0.68	0.52 ± 0.12	1.10	0.41	3.54	1.68 ± 1.34
C_{max} $\mu g/\ell$	20.19	25.36	21.67	22.41 ± 2.17	50.96	36.61	40.44	42.67 ± 6.07
t_{max} hr	3.97	4.49	4.18	4.21 ± 0.21	2.40	3.61	1.92	2.64 ± 0.71
k_{21} hr^{-1}	0.038	0.056	2.96	1.02 ± 1.37	0.026	0.029	0.090	0.048 ± 0.029
α hr^{-1}	0.17	0.21	3.53	1.30 ± 1.57	0.20	0.15	0.17	0.17 ± 0.021
λ_z hr^{-1}	0.0184	0.0190	0.0620	0.0331 ±0.020	0.0175	0.0101	0.0239	0.0172 ± 0.006
V ℓ/kg	33.87	27.42	32.32	31.20 ± 2.75	24.25	33.25	35.36	30.95 ± 4.82
•Cl ℓ/hr/kg	0.62	0.52	2.00	1.05 ± 0.68	0.42	0.34	0.85	0.54 ± 0.22

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_z x V

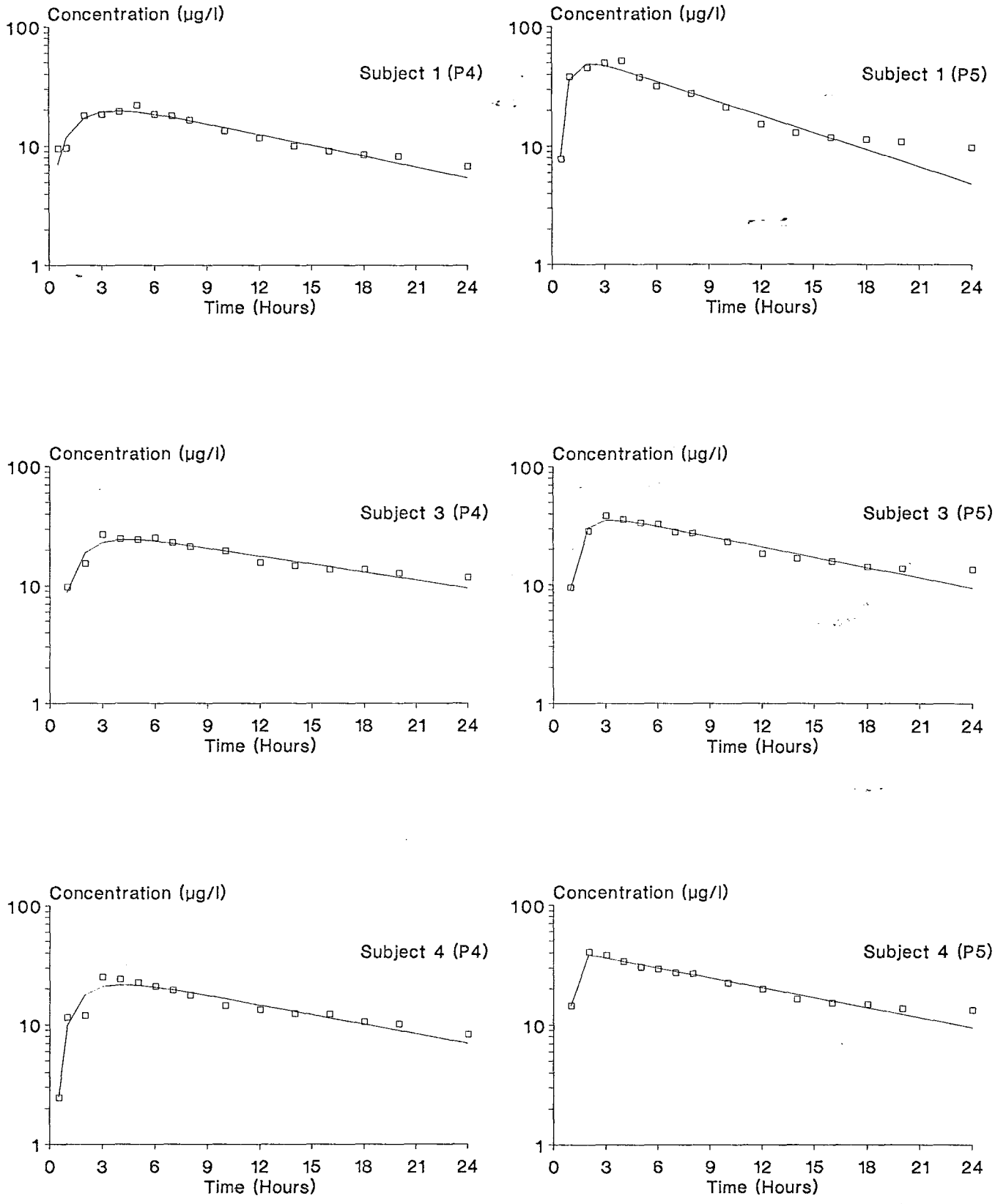


Figure 5.14 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1, 3 and 4 following administration of single oral doses of 100mg (P4) and 150mg (P5) of cyclizine hydrochloride. Model = PCNONLIN model 4 (1BCM).

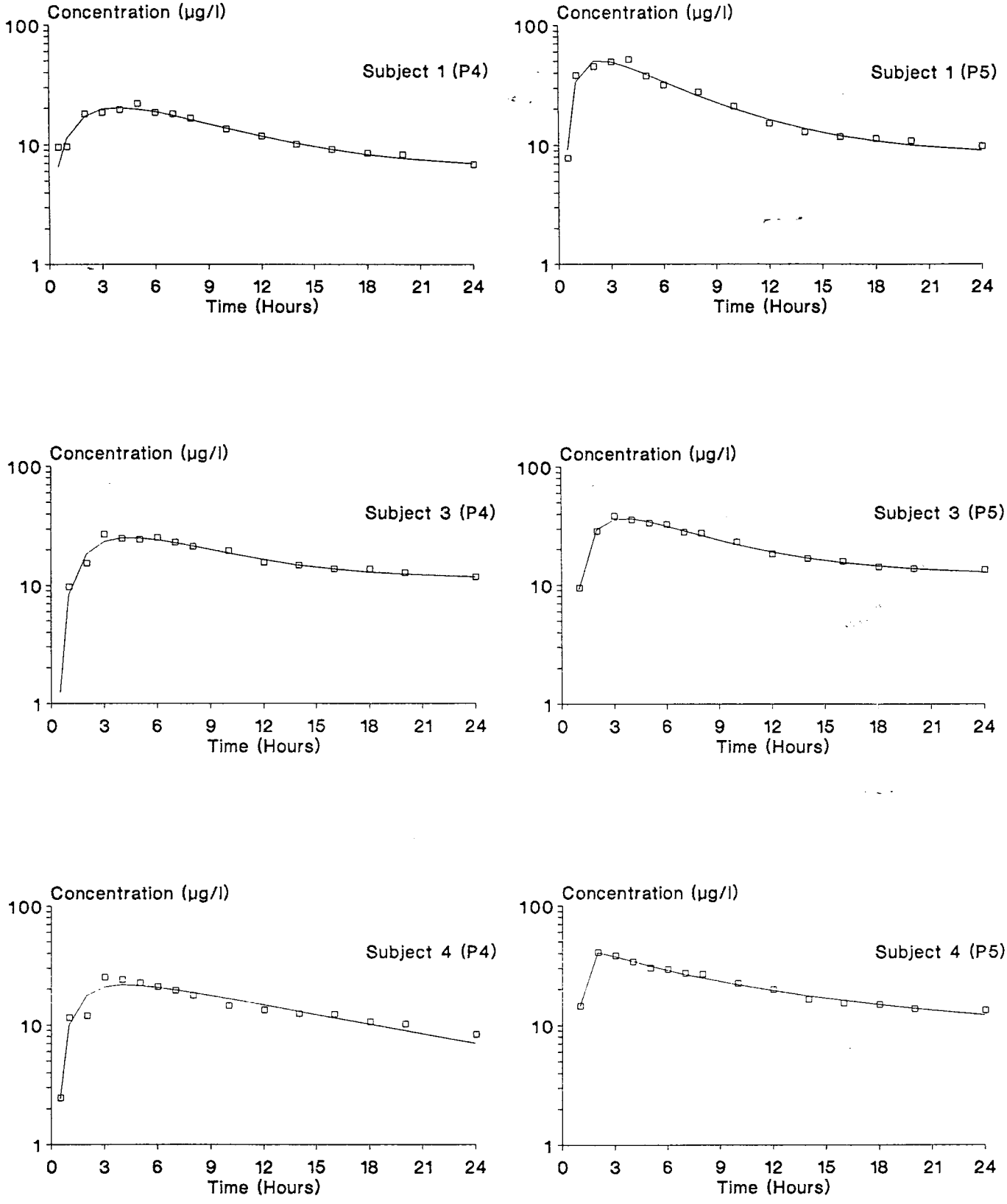


Figure 5.15 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1, 3 and 4 following administration of single oral doses of 100mg (P4) and 150mg (P5) cyclizine hydrochloride. Model = PCNONLIN model 12 (2BCM).

5.3.4.4 Multiple Dosing (Phase 3)

The results of fitting serum concentration time data to PCNONLIN Models 4 and 12 following multiple dosing of cyclizine hydrochloride are tabulated in Tables 5.17 (1BCM) and 5.18 (2BCM). Semilogarithmic plots of experimental and fitted serum concentration time data are depicted in Figures 5.16 (Model 4) and 5.17 (Model 12)

Table 5.17 Results of Compartment Modelling to PCNONLIN Model 4 (1BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following Administration of 50mg of Cyclizine Hydrochloride Every 8 Hours for Five Days.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	113.05	134.44	164.44	144.06	130.99	130.78	136.29 ± 15.57
**R^2	0.989	0.986	0.956	0.956	0.992	0.984	0.977 ± 0.0152
***SSR	96	244	900	370	210	208	338 ± 263
t_{lag} hr	1.99	0.65	0.50	0.54	0.27	1.49	0.91 ± 0.62
k_a hr^{-1}	7.48	1.95	2.51	0.98	1.43	1.98	2.72 ± 2.18
λ_z hr^{-1}	0.05	0.0652	0.0415	0.0421	0.0381	0.0452	0.0471 ± 0.009
V l/kg	34.71	17.02	27.74	36.54	23.64	34.34	28.99 ± 6.97
•Cl l/hr/kg	1.76	1.11	1.15	1.54	0.90	1.55	1.34 ± 0.30

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_z x V

Evaluation of goodness of fit data for compartment modelling of cyclizine serum concentration versus time data to both 1BCM and 2BCM following multiple doses reveal that the 2BCM provides a better description of the disposition of cyclizine. Comparison of parameters such as elimination rate, clearance and volume of distribution show a favourable correlation to those estimated previously using noncompartmental methods (Table 5.9).

Table 5.18 Results of Compartment Modelling to PCNONLIN Model 12 (2BCM) of Cyclizine Concentration Profiles for Subjects 1 to 6 Obtained Following Administration of 50mg of Cyclizine Hydrochloride Every 8 Hours for Five Days.

SUB	1	2	3	4	5	6	Mean ± S.D.
*AIC	86.90	104.44	166.34	141.25	112.11	131.62	123.78 ± 25.98
**R^2	0.997	0.996	0.960	0.967	0.997	0.986	0.983 ± 0.0150
***SSR	26	56	821	276	78	182	240 ± 273
t_{lag} hr	1.96	0.18	0.49	0.51	0.17	0.93	0.71 ± 0.62
k_a hr^{-1}	2.78	0.45	1.84	0.55	0.69	0.65	1.16 ± 0.86
k_{21} hr^{-1}	0.093	0.053	0.11	0.018	0.071	0.085	0.072 ± 0.029
α hr^{-1}	0.25	0.28	0.20	0.095	0.15	0.16	0.189 ± 0.062
λ_z hr^{-1}	0.0362	0.0280	0.0322	0.0114	0.0269	0.0347	0.0282 ± 0.008
V ℓ/kg	22.25	7.24	19.05	23.09	14.83	24.02	18.41 ± 5.86
•Cl ℓ/hr/kg	0.81	0.20	0.61	0.26	0.39	0.83	0.52 ± 0.25

* AIC = Akaike's information criteria (equation 5.26)
** R^2 = correlation coefficient
*** SSR = sum of squared residuals
• Cl = λ_z x V

5.3.4.5 Discussion

In summary, the intravenous data are best described by a three-compartment model and the oral dat by an open two-compartment model. Cyclizine appears to be rapidly and extensively distributed to the "shallow" peripheral compartment in the three-compartment model. Similarly, distribution to the peripheral compartment following oral administration is rapid and extensive. Distribution to the "deep" compartment is as expected slower than that to the "shallow" compartment. Cyclizine is intrinsically rapidly absorped and the rates of distribution to the central compartment (k_{21} and k_{31}) are much slower than the distribution rates (α, ß and γ) to the peripheral compartments and may contribute to a slower elimination rate if elimination occurs only via the central compartment. Elimination of the drug is slow with elimination half-lives following oral administration ranging from approximately 19 hours (50mg dose) to approximately 40 hours (150mg dose). The long $t_{½}$ following the 150mg dose is unlikely to be a result of saturation of enzyme systems or dose dependent pharmacokinetics (§ 5.3.2.2) but due to inherent errors in the use of models to predict features of a system (206). In addition, the reported pharmacokinetic parameters derived from modelling were obtained without weighting of data points. Clements *et al* (207) showed that pharmacokinetic parameters may be influenced considerably using weighting factors for individual data points and the use of such may provide better pharmacokinetic estimates for cyclizine. In addition, the use of simultaneous modelling of cyclizine and its metabolite may improve the estimation of pharmacokinetic parameters as was reported for albendazole (208).

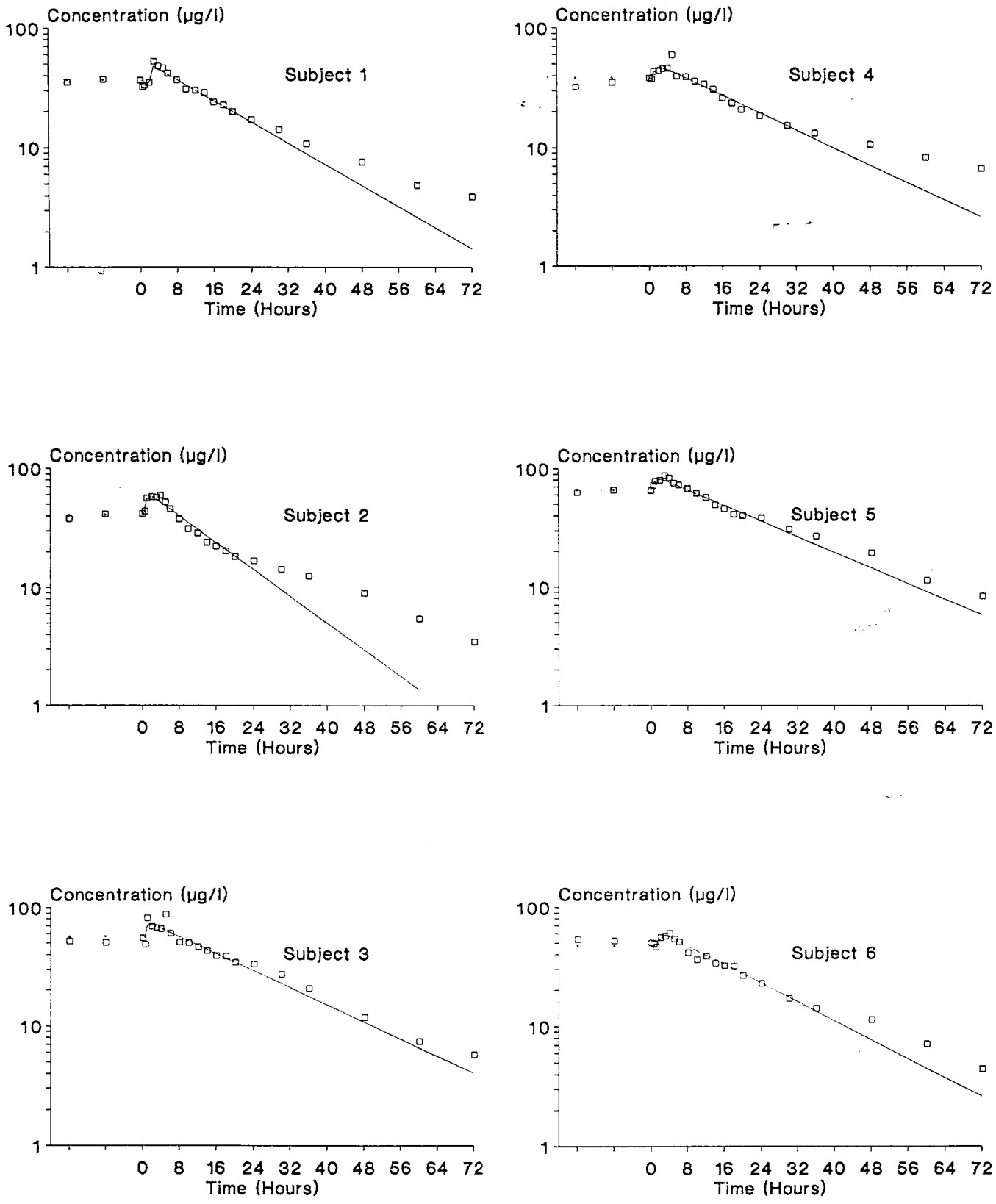


Figure 5.16 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of 50mg of cyclizine hydrochloride every 8 hours for five days, showing experimental (□) and fitted (■) trough concentrations before the 14th and 15th doses.
Model = PCNONLIN model 4 (1BCM).

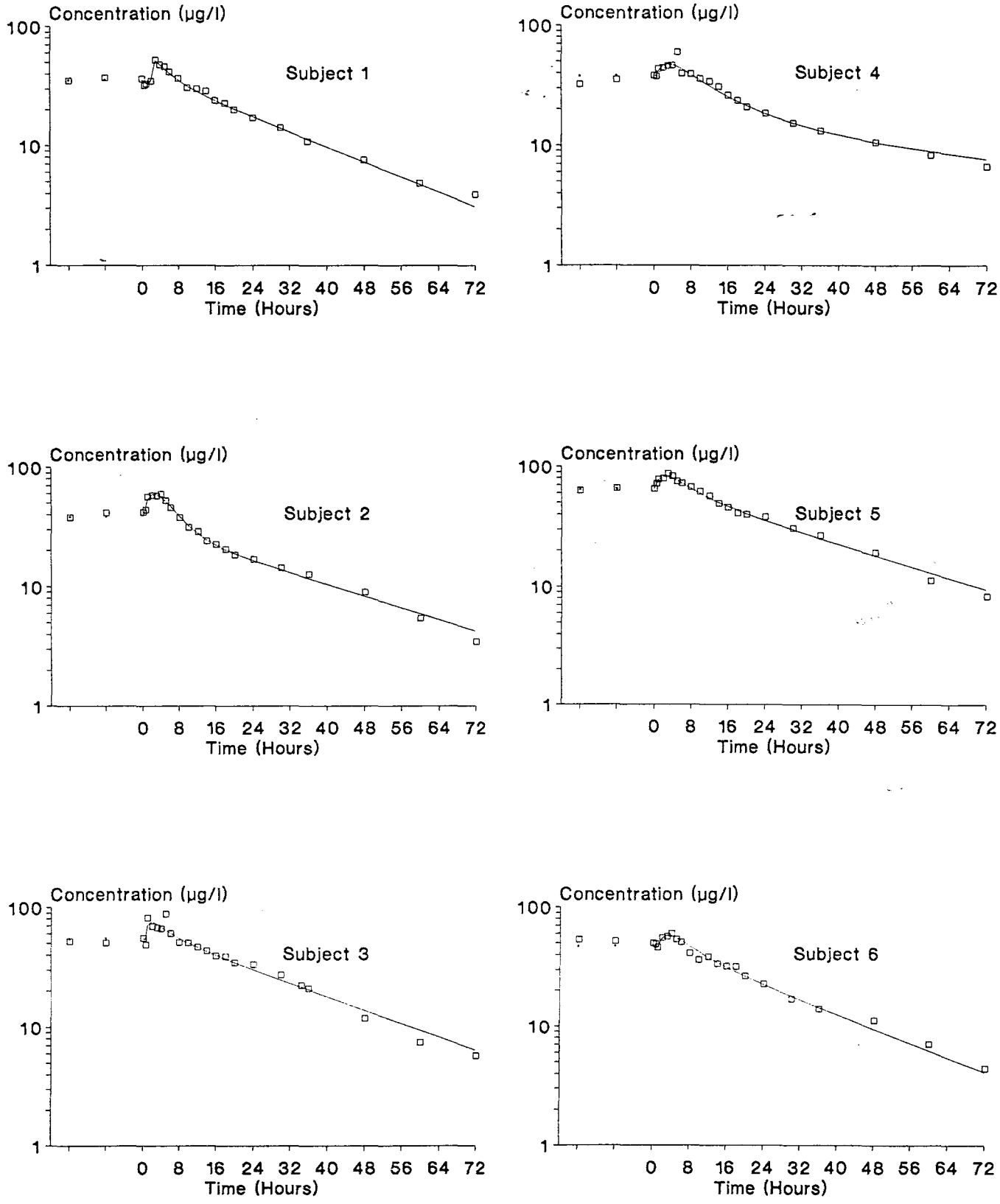


Figure 5.17 Semilogarithmic plots of experimental (□) and fitted data for cyclizine for subjects 1 to 6 following administration of 50mg of cyclizine hydrochloride every 8 hours for five days, showing experimental (□) and fitted (■) trough concentrations before the 14th and 15th doses.
Model = PCNONLIN model 12 (2BCM).

CHAPTER 6

CONCLUSION

There is a noticeable lack of pharmacokinetic studies and limited information on cyclizine in the literature, which is more than likely due to the intrinsic difficulties in determination of cyclizine at the low concentrations encountered in the systemic circulation following therapeutic doses of the drug. The extraction and HPLC method developed and described in this thesis has the necessary sensitivity, accuracy and precision for the selective determination of cyclizine and its demethylated metabolite, norcyclizine, in biological fluids and is thus suitable for the elucidation of the pharmacokinetics of the drug in humans. The application of this method to the analysis of blood samples following the administration of cyclizine to human volunteers as a single intravenous dose, oral doses of different dose sizes, and multiple oral doses enabled comprehensive pharmacokinetic characterization of this drug.

The results obtained following administration of a single intravenous and oral doses of cyclizine showed the drug to be approximately 50 percent bioavailable and attempts to predict average steady state concentrations resulted in an under prediction of these concentrations. Statistical comparisons of AUC^{∞} and C_{max} following increasing dose sizes of cyclizine to investigate possible dose-dependent pharmacokinetics of the drug showed that nonlinear kinetics are unlikely on repetitive dosing. Despite the small sample population used for the dose-dependent studies, nonlinear pharmacokinetics of cyclizine were not indicated. The apparent nonlinear increase in concentrations on repetitive dosing was probably related to the dosing frequency in accordance with recommendations of the manufacturer who suggest that a dose of 50mg of cyclizine be administered every 8 hours (41,209). Eight hours was seen to correspond to about one third of the half-life of 20 hours for cyclizine determined in the present studies. Consequently, dosing eight hourly resulted in accumulation of cyclizine and steady state concentrations in excess of what was expected based on single dose studies. The recommended dosing regimen was more than likely based on limited pharmacokinetic information obtained using analytical methods lacking in sensitivity to accurately quantitate the low concentrations of cyclizine found in the blood. As a result, the dosing interval may have been based on the distribution kinetics and not the elimination kinetics. The use of this dosing frequency, for administration of cyclizine to both adults and children may result in unnecessary accumulation of the drug with associated untoward side-effects. Cyclizine could more than likely be administered less frequently with beneficial therapeutic effects. These recommendations are similar to those made by Simons *et al* (53) who suggest that hydroxyzine, a piperazine H_1-receptor antagonist with an approximate half-life of 20 hours determined following a single oral dose and a

volume of distribution of 16ℓ/kg, would be as effective when administered once or twice daily as opposed to the recommended three or four times daily doses. In addition, experimental steady state concentrations for cyclizine were in close agreement with those computed using pharmacokinetic parameters elucidated from single dose studies, confirming that the disposition of cyclizine is adequately characterized by these parameters.
The availability of an intravenous dosage form enabled the calculation of absolute bioavailability, the volume of distribution and the total body clearance of cyclizine. In addition, urinary excretion data permitted the calculation of renal clearance of cyclizine.

Total clearance of the cyclizine was found to be low with the average Cl_{TOT} for single intravenous and oral studies in the region of 0.8ℓ/hr.kg. Renal clearance of cyclizine and norcyclizine were negligible with less than one percent of the dose excreted, thus emphasising that alternative mechanisms of elimination are responsible for the removal of cyclizine from the body. Differences in clearance between individual subjects may be a consequence of differences in metabolic pathways, other than the glucuronidation pathway (210). Urine samples were not hydrolysed, therefore, glucuronide derivatives of cyclizine that have been reported to account for elimination of approximately 15 percent of the administered dose were not isolated (73,74).

The average volume of distribution (V_Z) of cyclizine for all studies was extensive and in the region of approximately 20ℓ/kg. This value is 6 fold greater than that for methadone (211) and 2 fold that for digoxin (212), drugs considered to have large or extensive volumes of distribution. However, this large volume of distribution appears consistent with reports that H_1-receptor antagonists generally have extensive volumes of distribution ranging between 3.4ℓ/kg to 18.5ℓ/kg (213). Investigations into the protein and tissue binding of cyclizine might reveal additional information as to the causes of variability in the extent of distribution of the drug. Furthermore, knowledge pertaining to the rate and extent of formation of norcyclizine and other metabolites might enable a more accurate estimate of the volume of distribution and provide further information pertaining to elimination of cyclizine.

Serum concentration versus time data were fitted to open one-, two- and three-compartment models with the aid of PCNONLIN, a statistical analysis software package for nonlinear models. In general, the disposition of the drug appeared to be best described by the 3BCM for intravenous data and a 2BCM for oral data, based on criteria such as sum of squares, correlation coefficients and Aikaike's information criterion.

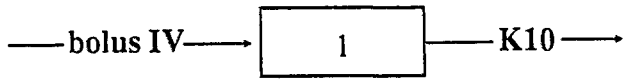
The use of more sophisticated models, in which the profiles of both parent drug and metabolite may be fitted simultaneously, or in which the intravenous data for cyclizine is fitted simultaneously with that for both parent drug and metabolite following oral dosing, may provide a better description of the disposition of cyclizine.

In summary, the use of HPLC with electrochemical detection for the analysis of cyclizine proved successful for the characterisation of the pharmacokinetics of the drug. Cyclizine is absorbed fairly rapidly with approximately 50 percent of the oral dose being bioavailable. Cyclizine, has a large volume of distribution, and is intrinsically slowly cleared from the biological system, via non-renal mechanisms. The long terminal elimination half-life of approximately 20 hours suggests that the drug would accumulate to steady-state only after several days. Information obtained from these studies has shed new light in respect of dosing recommendations especially when the drug is required for chronic use and should be of use in designing future systematic investigations on this drug.

APPENDIX 1

PCNONLIN MODELS USED FOR COMPARTMENTAL ANALYSIS (202)

MODEL 1. One compartment with bolus input and first-order output.



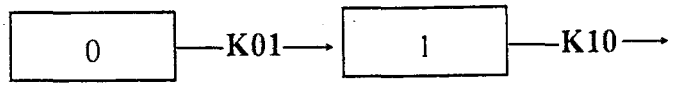
$$C(T) = D/V * EXP (K10*T)$$

Estimated parameters: (1) V = volume
(2) K10 = elimination rate

Constants in input: (1) # doses
(2) dose 1
(3) time of dose 1
(Repeat 2-3 for additional doses)

Secondary parameters: (1) AUC = D/V/K10
(2) K10 half-life
(3) CMAX = D/V

MODEL 4. One-compartment with first-order input, first-order output, and a lag time.



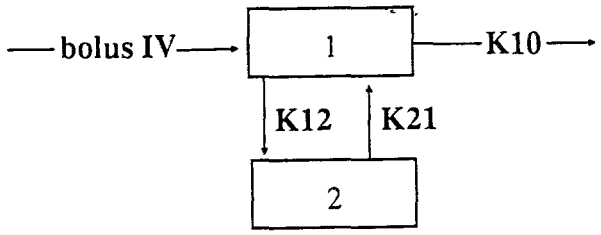
$$C(T) = D*K01/V/(K01-K10)*(EXP(-K10*T)-EXP(-K01*T))$$

Estimated parameters: (1) V = volume
(2) K01 = absorption rate
(3) K10 = elimination rate
(4) LT = lag time

Constants in input: (1) # doses
(2) dose 1
(3) time of dose 1
(Repeat 2-3 for additional doses)

Secondary parameters: (1) AUC = D/V/K10
(2) K01 half-life
(3) K10 half-life
(4) TMAX = ln (K01/K10)/(K01-K10)
(5) CMAX = C(TMAX)

MODEL 7. Two-compartment with bolus input and first-order output; micro-constants as primary parameters.



$$C(T) = A*EXP(-ALPHA*T) + B*EXP(-BETA*T)$$

where $A = D/V*(ALPHA-K21)/(ALPHA-BETA)$,
$B = -D/V*(BETA-K21)/(ALPHA-BETA)$,

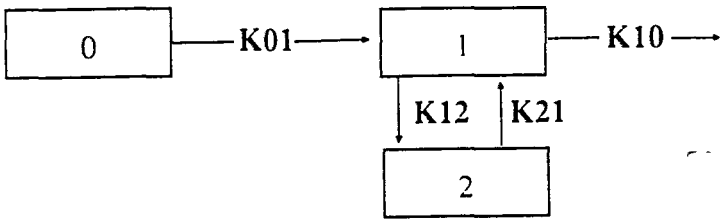
and ALPHA and BETA (ALPHA > BETA) are positive and negative roots of the quadratic equation
$(r*r + (K12 + K21 + K10)*r + K21*K10 = 0)$.

Estimated parameters:
(1) V = volume
(2) K10 = elimination rate
(3) K12 = transfer rate, 1 to 2
(4) K21 = transfer rate, 2 to 1

Contants in input:
(1) # doses
(2) dose 1
(3) time of dose 1
(Repeat 2-3 for additional doses)

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) ALPHA
(4) BETA
(5) ALPHA half-life
(6) BETA half-life
(7) A
(8) B
(9) CMAX = D/V

MODEL 12. Two-compartment with first-order input, first-order output, lag time and micro-constants as primary parameters.



$$C(T) = A*EXP(-ALPHA*T) + B*EXP(-BETA*T) + C*EXP(-K01*T),$$

where $A = D/V*K01*(K21-ALPHA)/(ALPHA-BETA)/(ALPHA-K01)$,
$B = -D/V*K01*(K21-BETA)/(ALPHA-BETA)/(BETA-K01)$,
$C = D/V*K01*(K21-K01)/(BETA-K01)/(ALPHA-K01)$,

and ALPHA and BETA (ALPHA > BETA) are positive and negative roots of the quadratic equation
$(r*r + (K12 + K21 + K10)*r + K21*K10 = 0)$.

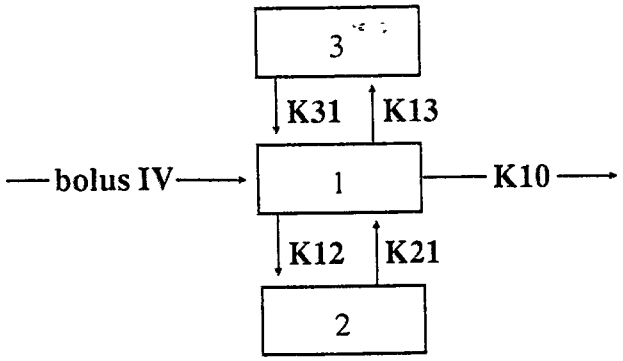
Estimated parameters:
(1) V = volume
(2) K01 = absorption rate
(3) K10 = elimination rate
(4) K12 = transfer rate, 1 to 2
(5) K21 = transfer rate, 2 to 1
(6) LT = lag time

Contants in input:
(1) # doses
(2) dose 1
(3) time of dose 1
(Repeat 2-3 for additional doses)

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) K01 half-life
(4) ALPHA
(5) BETA
(6) alpha half-life
(7) beta half-life
(8) A
(9) B
(10) Tmax*
(11) Cmax*

* Estimated for the compiled (internal) library only.

MODEL 18. Three-compartment with bolus input, first-order output and macro constants as primary parameters.



$$C(T) = A*EXP(-ALPHA*T) + B*EXP(-BETA*T) + C*EXP(-GAMMA*T)$$

Estimated parameters:
(1) A
(2) B
(3) C
(4) Alpha
(5) Beta
(6) Gamma

Constants at input:
(1) stripping dose
(2) # doses
(3) dose 1
(4) start time of dose 1
(Repeat 3-4 for additional doses)

Secondary parameters:
(1) Cmax
(2) Volume
(3) K21
(4) K31
(5) K10
(6) K12
(7) K13
(8) K10 half-life
(9) Alpha half-life
(10) Beta half-life
(11) Gamma half-life

REFERENCES

1. S.A. Benezra. Cyclizine. In K. Florey (ed.), *Analytical Profiles of Drug Substances*, **Volume 6**, Academic Press, New York, 1977, pp. 84-97.

2. *Martindale, "The Extra Pharmacopoeia"*, The Pharmaceutical Press, London, 29th Edition, London, 1989, pp. 443-446.

3. *The British Pharmacopoeia*, The Pharmaceutical Press, London, 1988, pp. 163-164.

4. *The United States Pharmacopoeia incorporating "The National Formulary"*, The United States Pharmacopoeial Convention Inc., XXII Edition, 1990, pp. 365-366.

5. *The Pharmaceutical Codex incorporating "The British Pharmaceutical Codex"*, The Pharmaceutical Press, London, 11th Edition, 1979, 236-237.

6. R. Baltzly, S. DuBreuil, W.S. Ide, and E. Lorz. Unsymmetrically disubstituted piperazines. III. N-methyl-N′-benzhydrylpiperazines as histamine antagonists. *J. Org. Chem.* **14**:775-781 (1949).

7. J.E. Driver. *Bentley and Driver's Textbook of Pharmaceutical Chemistry*, Oxford University Press, London, 7th Edition, 1960, pp. 566-567.

8. D.W. Newton, W.J. Murray, and M.W. Lovell. pK_a determination of benzhydrylpiperazine antihistamines in aqueous and aqueous methanol solutions. *J. Pharm. Sci.* **71**(12):1363-1366 (1982).

9. W.C. Bowman, and M.J. Rand, *Textbook of Pharmacology*, Blackwell Scientific Publications, London, 2nd Edition, 1980, pp. 6.20-6.21, 12.13-12.16, 26.23-26.24, 40.4.

10. V. Bertolasi, P.A. Borea, G. Gilli, and M. Sacerdoti. Cyclizine hydrochloride. *Acta Crystallogr. B.* **36**(8):1975-1977 (1980).

11. N. Sparrow. *Personal Communication*. (1985).

12. E.J. Ariens. Excursions into the field of SAR: A consideration of the past, the present, the future. In J.A. Keverling Buisman (ed.), *Biological Activity and Chemical Structure*, Elsevier Science Publishers B.V., Amsterdam, 1977, pp. 1-35.

13. V.J. Cirillo, and K.F. Tempero. Pharmacology and therapeutic use of antihistamines. *Am. J. Hosp. Pharm.* **33**:1200-1207 (1976).

14. E.A. Zepp, J.A. Thomas, and G.R. Knotts. Some pharmacologic aspects of the antihistamines: A survey of current clinical applications. *Clin. Pediat.* **14**(12):1119-1124 (1975).

15. W.W. Douglas. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In A. Goodman Gilman, L.S. Goodman, and A. Gilman (eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 6th Edition, The MacMillan Publishing Company Incorporated, New York, 1980, pp 609-646.

16. D.T. Witiak. Anti-allergenic agents. In A. Burger (ed.), *Medicinal Chemistry*, Wiley-Interscience, New York, 3rd Edition, Part II, 1970, 1643-1668.

17. C.A. Carter, N.J. Wojciechowski, J.M. Hayes, V.A. Skoutakis, and L.A. Rickman. Terfenadine: A nonsedating antihistamine. *Drug Intell. Clin. Pharm.* **19**:812-817 (1985).

18. D.T. Witiak, Z. Muhi-Eldeen, N. Mahishi, O.P. Sethi, and M.C. Gerald. L(*S*)- and D(*R*)-3-amino-1-phenylpyrrolidines: Stereo-selective antagonists for histamine and acetylcholine receptors *in vitro*. *J. Med. Chem.* **14**(1):24-30 (1971).

19. B. Potgieter. *Personal Communication*. (1991).

20. P.B. Marshall. Some chemical and physical properties associated with histamine antagonism. *Brit. J. Pharmacol.* **10**:270-278 (1955).

21. P. Turner, and A. Richens. *Clinical Pharmacology*, Churchill Livingstone Medical Text, London, 4th Edition, 1982, pp. 86-89.

22. R.S.J. Clarke, and J.W. Dundee. Side effects of anti-emetics: Results of a class experiment. *Eur. J. Pharmacol.* **14**:291-300 (1971).

23. H.I. Chinn, W.R. Gammon, and M.E. Frantz. Prevention of airsickness among airborne troops. *J. Appl. Physiol.* **5**:599-602 (1953).

24. R.M. Nicholl, J. Moore, and J.W. Dundee. Alterations in response to somatic pain associated with anaesthesia XI: Two non-phenothiazine anti-emetics: Cyclizine and trimethobenzamide. *Brit. J. Anaesth.* **34**:287-289 (1962).

25. V.K. Pendse, and B.R. Madan. Some pharmacological actions of cyclizine, chlorcyclizine and homchlorcyclizine. *Ind. J. Physiol. Pharmacol.* **13**(1):29-36 (1969).

26. A.C. Dreyer. Antihistamines-are you well informed? *S.A. Pharm. J.* **43**(10):366-373 (1976).

27. S. Norton, K.I. Colville, A.E. Light, A.L. Wnuck, R.V. Fanelli, and E.J. DE Beer. Pharmacologic properties of cyclizine hydrochloride (Marezine). *J. Pharmacol. Exp. Ther.* **112**:297-305 (1954).

28. F. Kerr, and K.W. Donald. Analgesia in myocardial infarction. *Brit. Heart J.* **36**:117-121 (1974).

29. L.B. Tan, S. Bryant, and R.G. Murray. Detrimental haemodynamic effects of cyclizine in heart failure. *The Lancet.* **1**(8585):560-561 (1988).

30. P.H. Gott. Cyclizine toxicity-Intentional drug abuse of a proprietary antihistamine. *New Eng. J. Med.* **279**:596 (1968).

31. E.P. Didier, T.G. Barila, H.C. Slocum, V.V. Lindgren, and E.L. McCawley. An evaluation of antiemetic drugs in the control of postoperative nausea and vomiting. *Surg. Forum Am. Coll.* 707-712 (1954).

32. G. Christie, S. Gersohn, R. Gray, F.H. Shaw, I. McCance, and D.W. Bruce. Treatment of certain side-effects of morphine. *Brit. Med. J.* **i**:675-678 (1958).

33. Hamilton, M., Bush, M. Bye, C. and Peck, A.W. A comparison of triprolidine and cyclizine on histamine (H_1) antagonism, subjective effects and performance tests in man. *Br. J. Clin. Pharmacol.* **13**:441-444 (1982).

34. J.C. Leza, I. Lizasoain, and P. Lorenzo. Effects of antihistaminics on locomotor activity in mice: Comparison with opiate and amphetamine-induced hyperactivity. *Gen. Pharmac.* **22**:293-296 (1991).

35. J.G. Brock-Utne, J.W. Downing, G.E. Dimopoulos, and M.G. Moshal. The effect of cyclizine hydrochloride on lower oesophageal sphincter pressure in man. *S. Afr. Med. J.* **51**:977-978 (1977).

36. J.G. Brock-utne, and J.W. Downing. The lower oesophageal sphincter and the anaesthetist. *S. Afr. Med. J.* **70**:170-171 (1986).

37. A.S.E. Fowle, D.T.D. Hughes, and G.J. Knight. The evaluation of histamine antagonists in man. *Eur. J. Clin. Pharmacol.* **3**:215-220 (1971).

38. J.W. Dundee, F. Halliday, R.M. Nicholl, and J. Moore. Studies of drugs given before anaesthesia. X: Two non-phenothiazine anti-emetics - cyclizine and trimethobenzamide. *Br. J. Anaesth.* **38**:50-57 (1966).

39. J.J. Brand, and W.L.M. Perry. Drugs used in motion sickness: A critical review of the methods available for the study of drugs of potential value in its treatment and of the information which has been derived by these methods. *Pharmacol. Rev.* **18**(1):895-924 (1966).

40. R.E. Westcombe, and R.K.J. Price. Meptazinol in the casualty department: A comparison against morphine. *Curr. Ther. Res. Clin. Exp.* **37**(5):969-979 (1985).

41. Valoid: Product Information Leaflet. Wellcome (Pty) Limited, Johannesburg, Republic of South Africa.

42. D.M. Paton, and D.R. Webster. Clinical pharmacokinetics of H_1-receptor antagonists (The Antihistamines). *Clin. Pharmacokinet.* **10**:477-497 (1985).

43. R. Kuntzman, A. Klutch, I. Tsai, and J.J. Burns. Physiological distribution and metabolic inactivation of chlorcyclizine and cyclizine. *J. Pharmacol. Exp. Ther.* **149**(1):29-35 (1965).

44. G.C. Mueller, and J.A. Miller. The metabolism of methylated aminoazo dyes: II. Oxidative demethylation by rat liver homogenates. *J. Biol. Chem.* **202**:579-587 (1953).

45. F.E. Simons, K.J. Simons, M. Chung, and J. Yeh. The comparative pharmacokinetics of H_1-receptor antagonists. *Ann. Allergy.* **59**(II): 20-24 (1987)

46. D.S. Griffin, and R.C. Baselt. Blood and urine concentrations of cyclizine by nitrogen-phosphorus gas-liquid chromatography. *J. Anal. Toxicol.* **8**:97-99 (1984).

47. R.B. Walker, and I. Kanfer. High-performance liquid chromatographic analysis of cyclizine and its metabolite, norcyclizine in biological fluids using solid-phase extraction. *Chromatographia.* **24**:287-290 (1987).

48. R.C. Backer, P. McFeeley, and N. Wohlenberg. Fatality resulting from cyclizine overdose. *J. Anal. Toxicol.* **13**: 308-309 (1989).

49. M. Puttemans, M. Bogaert, G. Hoogewijs, L. Dryon, D.L. Massart, and L. Vanhaelst. Determination of cinnarizine in whole blood and plasma by reversed phase HPLC and it application to a pharmacokinetic study. *J. Liq. Chromatogr.* **7**(11):2237-2250 (1984).

50. P.J. Morrison, I.D. Bradbrook, and H.J. Rogers. Plasma cinnarizine levels resulting from the oral administration as capsule or tablet formulation investigated by gas-liquid chromatography. *Br. J. Clin. Pharmac.* 7:349-352 (1979).

51. H.K.L. Hundt, L.W. Brown, and E.C. Clarck, Determination of cinnarizine in plasma by high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* **183**:378-382 (1980).

52. H.G Fouda, D.C. Hobbs, and J.E. Stambaugh. Sensitive assay for determination of hydroxyzine in plasma and its human pharmacokinetics. *J. Pharm. Sci.* **68**(11):1456-1458 (1979).

53. F.E.R. Simons, K.J. Simons, and E.M. Frith. The pharmacokinetics and antihistaminic of the H_1-receptor antagonist hydroxyzine. *J. Allergy Clin. Immunol.* **73**(1):69-75 (1984).

54. F.E.R. Simons, K.J. Simons, A.B. Becker, and R.P. Haydey. Pharmacokinetics and antipruritic effects of hydroxyzine in children with atopic dermatitis. *J. Pediatr.* **104**(1):123-127 (1984).

55. K.J. Simons, W.T.A. Watson, X.Y. Chen, and F.E.R. Simons. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly. *Clin. Pharmacol. Ther.* **45**(1):9-14 (1989).

56. F.E.R. Simons, W.T.A. Watson, X.Y. Chen, G.Y. Minuk, and K.J. Simons. The pharmacokinetics and pharmacodynamics of hydroxyzine in patients with primary biliary cirrhosis. *J. Clin. Pharmacol.* **29**:809-815 (1989).

57. H.G. Fouda, F.C. Falkner, D.C. Hobbs, and E.W. Luther. Selected ion monitoring assay for meclizine in human plasma. *Biomed. Mass Spectrom.* **5**(8):491-494 (1978).

58. T. Imai, S. Kimura, T. Imamura, K. Uchiyama, T. Iijima, W.J. Underberg, and M. Otagiri. Quantitation of meclizine dihydrochloride in serum by reversed phase ion-pair high-performance liquid chromatography. *J. Liq. Chromatogr.* **10**(11):2205-2512 (1987).

59. J.P. Chovan, R.P. Klett, and N. Rakieten. Comparison of meclizine levels in plasma of rats and dogs after intranasal, intravenous and oral administration., *J. Pharm. Sci.* **74**(10):1111-1113 (1985).

60. G. Land, K. Dean, and A. Bye. Determination of cyclizine and norcyclizine in plasma and urine using gas-liquid chromatography with nitrogen selective detection. *J. Chromatogr. Biomed. Appl.* **222**:134-140 (1981).

61. T. Tokumura, Y. Tsushima, M. Kayano, Y. Machida, and T. Nagai. Enhancement of bioavailability of cinnarizine from its β-cyclodextrin complex on oral administration with DL-phenylalanine as a competing agent. *J. Pharm. Sci.* **74**(4):496-497 (1985).

62. T. Tokumura, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida, and T. Nagai. Enhancement of the oral bioavailability of cinnarizine in oleic acid in beagle dogs. *J. Pharm. Sci.* **76**(4):285-288 (1987).

63. H.J. Gaertner, U. Breyer, and G. Liomin. Chronic administration of chlorcyclizine and meclizine to rats: Accumulation of a metabolite formed by piperazine ring cleavage. *J. Pharmacol. Exp. Ther.* **185**(2):195-201 (1973).

64. W.J. Waddell. The distribution of chlorcyclizine-^{14}C in pregnant mice. *Drug Metab. Dispos.* **1**(3):598-601 (1973).

65. S.F. Pong, and C.L. Huang. Comparative studies on distribution, excretion, and metabolism of hydroxyzine-^{3}H and its methiodide-^{14}C in rats. *J. Pharm. Sci.* **63**(10):1527-1531 (1974).

66. K.J. Simons, and F.E.R. Simons. The effect of chronic administration of hydroxyzine on hydroxyzine pharmacokinetics in dogs. *J. Allergy Clin. Immunol.* **79**(6):928-932 (1987).

67. H.W. Jun, N.H. Foda, and J.W. McCall. Reversed-phase liquid chromatographic determination of meclozine in biological samples. *J. Pharm. Biomed. Anal.* **5**(3):295-301 (1987).

68. W.A. Ritschel. *Handbook of basic pharmacokinetics ... including clinical applications*, Drug Intelligence Publications Incorporated, Hamilton, 4th Edition, 1992, pp. 213-217.

69. M. Gibaldi. *Biopharmaceutics and Clinical Pharmacokinetics*, Lea & Febiger, Philadelphia, 3rd Edition, 1984, pp. 191-195.

70. Burns, J.J., Conney, A.H. and Koster R. Stimulatory effect of chronic drug administration on drug-metabolising enzymes in liver microsomes. *Ann. N.Y. Acad. Sci.* **104**:881-893 (1963).

71. R. Kuntzman, A. Phillips, I. Tsai, A. Klutch, and J.J. Burns. N-oxide formation: A new route for inactivation of the antihistaminic chlorcyclizine. *J. Pharmacol. Exp. Ther.* **155**(2):337-344 (1967).

72. M. Nishikata, A. Nakai, H. Fushida, K. Miyake, T. Arita, K. Iseki, K. Miyazaki, and A. Nomura. Enantioselective pharmacokinetics of homchlorcyclizine. III. Simultaneous determination of (+)- and (-)- homchlorcyclizine in human urine by high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* **612**:239-244 (1993).

73. H. Luo, E.M. Hawes, G. McKay, E.D. Korchinski, and K.K. Midha. N^{+}-glucuronidation of aliphatic tertiary amines, a general phenomenon in the metabolism of H_1-antihistamines in humans. *Xenobiotica.* **21**:1281-1288 (1991).

74. H. Luo, E.M. Hawes, G. McKay, E.D. Korchinski, and K.K. Midha. The quaternary ammonium-linked glucuronide metabolite of cyclizine is excreted in human faeces. *Pharm. Res. Suppl.* **9**(10):S-325 (1992).

75. F.M. Gengo, J. Dabronzo, A. Yurchak, S. Love, and J.K. Miller. The relative antihistaminic and psychomotor effects of hydroxyzine and cetirizine. *Clin. Pharmacol. Ther.* **42**:265-272 (1987).

76. S.A. Narrod, A.L. Wilk, and C.T.G. King. Metabolism of meclizine in the rat. *J. Pharmacol. Exp. Ther.* **147**(3):380-384 (1965).

77. A.H. Conney, and A. Klutch. Increased activity of androgen hydroxylases in liver microsomes of rats pretreated with phenobarbital and other drugs. *J. Biol. Chem.* **238**(5):1611-1617 (1963).

78. M. Rowland, and T.N. Tozer. *Clinical Pharmacokinetics: Concepts and Applications*, Lea & Febiger, Philadelphia, 2nd Edition, 1989, p. 149.

79. R. Kuntzman, I. Tsai, and J.J. Burns. Importance of tissue and plasma binding in determining the retention of norchlorcyclizine and norcyclizine in man, dog and rat. *J. Pharmacol. Exp. Ther.* **158**(2):332-339 (1967).

80. R. Kuntzman, M. Jacobson, I. Tsai and J.J. Burns. Certain aspects of drug binding to non-plasma proteins as illustrated by studies with cyclizine, chlorcyclizine and polymyxin B. *Ann. N. Y. Acad. Sci.*, **226**:131-147 (1973).

81. J.A. Thompson, D.C. Bloedow, and F.H. Leffert. Pharmacokinetics of intravenous chlorpheniramine in children. *J. Pharm. Sci.* **70**(11):1284-1286 (1981).

82. E.E. Jensen, and R.T. Pflaum. Fluorometric Study of Antihistamines. *J. Pharm. Sci.* **53**(7):835-837 (1964).

83. A. MacDonald Jnr., R.T. Pflaum. Gas chromatography of some antihistamines. J. Pharm. Sci. 53(8):887-891 (1964).

84. R.T. Sane, and U.M. Vaidya. Colorimetric estimation of cyclizine. *Indian J. Pharm. Sci.* **41**(2):73-74 (1979).

85. M.J.M. Campbell, B. Demetriou, and R. Jones. Assay of procyclidine hydrochloride, cyclizine hydrochloride and diethylcarbazine citrate in tablets using ion-responsive electrodes. *Analyst* **105**:605-611 (1980).

86. A.G. Davidson, and S.M. Hassan. Assay of benzoid drugs in tablet and capsule formulations by second-derivative ultraviolet spectrophotometry. *J. Pharm. Sci.* **73**(3):413-416 (1984).

87. G.K.C. Low, P.R. Haddad, and A.M. Duffield. Analysis of some commercial preparations for migraine treatment using ion-pair high-performance liquid chromatography with addition of salts to the mobile phase. *J. Chromatogr.* **261**:345-356 (1983).

88. I.M. Jalal, S.I. Sa'sa', and T.A. Yasin. Determination of ergotamine tartrate and cyclizine hydrochloride in pharmaceutical tablets by reversed phase HPLC. *Anal. Letters* **21**(9):1561-1577 (1988).

89. I. Kanfer, and N.A. Sparrow. *HPLC determination of cyclizine and its metabolite, norcyclizine in serum.* Presented at APhA Academy of Pharmaceutical Sciences Annual Meeting, Montreal, Canada (1984).

90. L.R. Snyder, and J.J. Kirkland. *Introduction to Modern Liquid Chromatography.* Wiley-Interscience, New York, 1979.

91. A.C. Mehta. Preanalytical considerations in drug assays in clinical pharmacokinetic studies. *J. Clin. Pharm. Ther.* **14**:285-295 (1989).

92. S. Ahuja. Selectivity and detectability optimizations in HPLC. In J.D. Winefordner and I.M. Kolthoff (eds.), *Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications*, **Volume 104**, John Wiley and Sons, New York, 1989, p. 2.

93. G. M. Pollack. Chromatographic analysis. In J. Swarbrick and J.C. Boylan (eds.), *Encyclopedia of Pharmaceutical Technology,* **Volume 2**, Marcel Dekker, Inc., New York, 1987, p. 439.

94. R.E. Majors. Practical operations of bonded-phase columns in high-performance liquid chromatography. In C. Horváth (ed.), *High-Performance Liquid Chromatography: Advances*

and Perspectives, **Volume 1**, Academic Press, New York, 1980, pp. 75-111.

95. P. Roumeliotis and K.K. Unger. Structure and properties of *n*-alkyldimethylsilyl bonded silica reversed-phase packings. *J. Chromatogr.* **149**:211-224 (1978).

96. J.W. Cox, and R.H. Pullen. Irregular retention properties of 21-aminosteroid antioxidants in octylsilane bonded-phase high-performance liquid chromatography. *J. Chromatogr.* **424**: 285-292 (1988).

97. M.J. Walters. Classification of octadecyl-bonded liquid chromatography systems. *J. Assoc. Off. Anal. Chem.* **70**(3):465-469 (1987).

98. P.J. Twitchwett and A.C. Moffat. High-pressure liquid chromatography of drugs. An evaluation of an octadecylsilane stationary phase. *J. Chromatogr.* **111**:149-157 (1975).

99. W.R. Melander and C. Horváth. Reversed-phase chromatography. In C. Horváth (ed.), *High-Performance Liquid Chromatography: Advances and Perspectives*, **Volume 2**, Academic Press, New York, 1980, pp. 145-148.

100. P.J. Schoenmakers. *Optimization of Chromatographic Selectivity. A guide to method development.* Journal of Chromatography Library, **Volume 35**, Elsevier, Amsterdam, 1986, p. 9.

101. K. Hammarstrand. Internal standard in gas chromatography. *Varian Instrument Applications.* **10**(1):10-11 (1976).

102. N. Simpson. Solid-phase extraction. Disposable chromatography. *Int. Chromatogr. Lab.* **11**:7-13 (1992).

103. R.D. McDowall, J.C. Pearce, and G.S. Murkitt. Liquid-solid sample preparation in drug analysis. *J. Pharm. Biomed. Anal.* **4**(1):3-21 (1986).

104. K.C. Van Horne (ed.), *Handbook of Sorbent Extraction Technology*, Analytichem International Inc., Harbor City, 1985.

105. R.D. McDowall, E. Doyle, G.S. Murkitt, and V.S. Picot. Sample preparation for the HPLC analysis of drugs in biological fluids. *J. Pharm. Biomed. Anal.* **7**(9):1087-1096 (1989).

106. B. Tippins. Selective sample preparation of endogenous biological compounds using solid-phase extraction. *International Laboratory* **17**(3):28-36 (1987).

107. C.M. Moore. Solid-phase cation exchange extraction of basic drugs from the urine of racing greyhounds. *J. Foren. Sci. Soc.* **30**(3):123-129 1990.

108. C. Stubbs, J.M. Haigh, and I. Kanfer. Determination of erythromycin in serum and urine by high-performance liquid chromatography with ultraviolet detection. *J. Pharm. Sci.* **74**(10): 1126-1128 (1985).

109. C. Stubbs, J.M. Haigh, and I. Kanfer. High-performance liquid chromatographic analysis of oleandomycin in serum and urine. *J. Chromatogr.* **353**:33-38 (1986).

110. M. Skinner, and I. Kanfer. High-performance liquid chromatographic analysis of josamycin in serum and urine. *J. Chromatogr.* **459**:261-267 (1988).

111. J. Chamberlain. *Analysis of Drugs in Biological Fluids.* CRC Press Inc., Boca Raton, 1985, pp. 27-31, 156-160.

112. E. Reid. Sample preparation in the micro-determination of organic compounds in plasma or urine. *Analyst* **101**:1-18 (1976).

113. J.C. Mathies, and M.A. Austin. Modified acetonitrile protein-precipitation method of sample preparation for drug assay by liquid chromatography. *Clin. Chem.* **26**(12):1760 (1980).

114. C.K. Lim. Sample preparation for high-performance liquid chromatography in the clinical laboratory. *Tr. Anal. Chem.* **7**(9):340-345 (1988).

115. M.F. Skinner. *Biopharmaceutics and Pharmacokinetics of the Macrolide Antibiotic - Josamycin.* PhD. Thesis, Rhodes University, December 1991, pp. 57.

116. R.J. Ruane, and I.D. Wilson. The use of C18 bonded silica in the solid-phase extraction of basic drugs - possible role for ionic interactions with residual silanols. *J. Pharm. Biomed. Anal.* **5**(7):724-727 (1987).

117. B. Law, S. Weir, and N.A. Ward. Fundamental studies in reversed-phase liquid-solid extraction of basic drugs; I: ionic interactions. *J. Pharm. Biomed. Anal.* **10**(2/3):167-179 (1992).

118. B. Law, and S. Weir. Fundamental studies in reversed-phase liquid-solid extraction of basic drugs; II: hydrogen bonding effects. *J. Pharm. Biomed. Anal.* **10**(2/3):181-186 (1992).

119. B. Law, and S. Weir. Fundamental studies in reversed-phase liquid-solid extraction of basic drugs; III: sample matrix effects. *J. Pharm. Biomed. Anal.* **10**(7):487-493 (1992).

120. G.A. Junk, M.J. Avery, and J.J. Richard. Interferences in solid-phase extraction using C-18 bonded porous silica cartridges. *Anal. Chem.* **60**(13):1347-1350 (1988).

121. C. Hartmann, D.L. Massart, and R.D. McDowall. An analysis of the Washington Conference Report on bioanalytical method validation. *J. Pharm. Biomed. Anal.* **12**(11):1337-1343 (1994).

122. J.J. De Ridder. Accuracy and Precision in GC-MS quantitation. In E. Reid (ed.), *Blood Drugs and Other Analytical Challenges,* Ellis Horwood, Chichester, 1978, p. 153.

123. G.W. Peng, and W.L. Chiou. Analysis of drugs and other toxic substances in biological samples for pharmacokinetic studies. *J. Chromatogr. Biomed. Appl.* **531**:3-50 (1990).

124. M.A. Brooks, and E.E. Weinfeld. A validation process for the data from the analysis of drugs in biological fluids. *Drug Dev. Ind. Pharm.* **11**(9&10):1703-1728 (1985).

125. T.M. Ludden. *Estimation of limit of quantitation.* Presented at F.I.P. BIO-International '94, Bioavailability, Bioequivalence and Pharmacokinetic Studies, Munich, Germany (1994).

126. A.R. Buick, M.V. Doig, S.C. Jeal, G.S. Land, and R.D. McDowall. Method validation in the bioanalytical laboratory. *J. Pharm. Biomed. Anal.* **8**(8-12):629-637 (1990).

127. P.J. Howard. The stability of diazepam in plasma samples when stored under varying conditions. *J. Pharm. Pharmac.* **30**:136 (1978).

128. J.D. O'Broin, I.J. Temperley, and J.M. Scott. Erythrocyte, plasma, and serum folate: Specimen stability before microbial assay. *Clin. Chem.* **26**(3):522-524 (1980).

129. D. Lissner, R.S. Mason, and S. Posen. Stability of vitamin D metabolites in human blood serum and plasma. *Clin. Chem.* **27**(5):773-774 (1981).

130. J.H.G. Jonkman, J.P. Franke, R. Schoenmaker, and R.A. de Zeeuw. Stability of theophylline in serum, plasma, and saliva stored at different temperatures. *Clin. Chem.* **27**(12):2071-2072 (1981).

131. U. Timm, M. Wall, and D. Dell. A new approach to dealing with the stability of drugs in biological fluids. *J. Pharm. Sci.* **74**(9):972-977 (1985).

132. J.W. Munson. High-performance liquid chromatography: Theory, instrumentation, and pharmaceutical applications. In J.W. Munson (ed.), *Drugs and the Pharmaceutical Sciences: Pharmaceutical Analysis Modern Methods*, **Volume 11**, Part B, Marcel Dekker, Inc., New York, 1984, pp. 15-154.

133. P.T. Kissinger. Electrochemical detection in liquid chromatography and flow injection analysis. In P.T. Kissinger and, W.R. Heineman (eds.), *Laboratory Techniques in Electroanalytical Chemistry*, Marcel Dekker, Inc., New York, 1984, pp. 611-635.

134. L.J. Lorenz. High-performance liquid chromatography. In R.E. Schirmer (ed.), *Modern Methods of Pharmaceutical Analysis*, **Volume 3**, CRC Press Inc., Boca Raton, 1982, pp. 61-137.

135. T. Nagatsu, and K. Kojima. Application of electrochemical detection in high-performance liquid chromatography to the assay of biologically active compounds. *Tr. Anal. Chem.* **7**(1):21-27 (1988).

136. R.P.W. Scott. *Liquid Chromatography Detectors.* Journal of Chromatography Library, **Volume 33**, Elsevier, Amsterdam, 1986, pp. 117-123.

137. L.H. Fleming, J.P. Milsap, and N.C. Reynolds Jnr. Recognizing and eliminating noise problems in liquid chromatography. *LC-GC* **6**(11):978-985 (1988).

138. *Coulochem Instruction Manual.* 5th Edition, Environmental Sciences Associates, Inc., Bedford, Massachusetts, U.S.A., January 1983.

139. C. Stubbs. *Application of High-performance Liquid Chromatography to the Analysis, Stability and Pharmacokinetics of Erythromycin.* PhD. Thesis, Rhodes University, January 1988.

140. F. Glew. *High-performance Liquid Chromatographic Studies of the Acid Degradation, Pharmacokinetics and Comparative Bioavailability of Erythromycin.* MSc. Thesis, Rhodes University, December 1988.

141. S. Terespolsky. *A Study of the Biopharmaceutics and Pharmacokinetics of the Macrolide Antibiotic, Erythromycin.* MSc. Thesis, Rhodes University, January 1991.

142. M. Goto, E. Sakurai, and D. Ishii. Dual electrochemical detection of biogenic amine metabolites in micro high-performance liquid chromatography. *J. Liq. Chromatogr.* **6**(10):1907-1925 (1983).

143. J. Descombe, G. Doumont, and M. Picard. Determination of exifone in human plasma and urine by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. Biomed. Appl.*, **496**:345-353 (1989).

144. C.F. McLean, L.E. Mather, J. Odontiadis, and P.A. Sloan. Improved method for morphine determination in biological fluids and tissues: rapid, sensitive and selective. *J. Pharm. Pharmacol.* **42**:669-671 (1990).

145. R. Whelpton, P. Dudson, H. Cannell, and K. Webster. Determination of prilocaine in human plasma samples using high-performance liquid chromatography with dual-electrode electrochemical detection. *J. Chromatogr. Biomed. Appl.* **526**:215-222 (1990).

146. L. Embree, and K.M. McErlane. Electrochemical detection of the 3,5-dinitrobenzoyl derivative of digoxin by high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* **526**:439-446 (1990).

147. P. Betto, A. Meneguz, G. Ricciarello, and S. Pichini. Simultaneous high-performance liquid chromatographic analysis of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in plasma using electrochemical detection. *J. Chromatogr. Biomed. Appl.* **575**:117-121 (1992).

148. B. Grobler. *Synthesis and Pharmacological Evaluation of the Antiarrhythmic Activity of Structurally Related Analogues of Procainamide.* MSC. Thesis, University of Port Elizabeth, March 1992.

149. J.W. Cox, and R.H. Pullen. High-performance liquid chromatographic determination of a 21-aminosteroid antioxidant in plasma. *J. Chromatogr. Biomed. Appl.* **424**:293-302 (1988).

150. P. De Leenheer, and H.J.C.F. Nelis. Development and evaluation of selected assays for drugs and drug metabolites in biological materials. *Analyst* **106**(1267):1025-1035 (1981).

151. J.L. Ford. Dissolution and dissolution testing. In J. Swarbrick and J.C. Boylan (eds.), *Encyclopedia of Pharmaceutical Technology,* **Volume 4**, Marcel Dekker, Inc., New York, 1991, pp. 121-167.

152. U.V. Banakar. Introduction, historical highlights, and the need for dissolution testing. In J. Swarbrick (ed.), *Drugs and the Pharmaceutical Sciences: Pharmaceutical Dissolution Testing,* **Volume 49**, Marcel Dekker, Inc., New York, 1992, pp.1-51.

153. V.P. Shah, J. Elkins, S. Lam, and J.P. Skelly. Determination of the in vitro release from hydrocortisone creams. *Int. J. Pharm.* **56**(1):53-59 (1989).

154. M. Pernarowski. Dissolution Methodology. In L.J. Leeson and J.T. Carstensen (eds.), *Dissolution Technology,* The Industrial Pharmaceutical Technology Section of the Academy of Pharmaceutical Sciences, Washington D.C., U.S.A., 1974, pp. 58-105.

155. J.G. Wagner. Measurement of *in vitro* rates of dissolution from capsules and tablets. In *Biopharmaceutics and Relevant Pharmacokinetics,* Drug Intelligence Publications Incorporated, Hamilton, 1st Edition, 1971, pp. 110-114.

156. J.W. McGinty, S.A. Stavchansky, and A. Martin. Bioavailability and tablet technology. In H.A. Lieberman and L. Lachman (eds.), *Pharmaceutical Dosage Forms: Tablets,* **Volume 2,** Marcel-Dekker, Inc., New York, 1981, pp. 269-449.

157. A. Dakkuri, and A.S. Shah. Dissolution methodology. *Pharm. Tech.* 6(6):28-86 (1982).

158. *The British Pharmacopoeia*, The Pharmaceutical Press, London, 1993, pp. A160-A162.

159. *The United States Pharmacopoeia incorporating "The National Formulary"*, The United States Pharmacopoeial Convention Inc., XXIII Edition, 1995, pp. 1791-1799.

160. R.K. Baweja. Dissolution testing of oral solid dosage forms using HPLC. *Pharm. Tech.* **11**(1):28-36 (1987).

161. K. Chan. Pharmacokinetic contributions to drug use. *J. Clin. Hosp. Pharm.* **10**:257-267 (1985).

162. J.M. van Rossum, J. Burgers, G. van Lingen, and J. de Bie. Pharmacokinetics: A dynamic systems approach. *Tr. Pharmacol. Sci.* **4**(1):27-30 (1983).

163. C.K. Svensson. Ethical considerations in the conduct of clinical pharmacokinetic studies. *Clin. Pharmacokinet.* **17**(4):217-222 (1989).

164. R.E. Notari, *Biopharmaceutics and Clinical Pharmacokinetics: An Introduction*, Marcel Dekker Inc., New York, 4th Edition, 1987.

165. L. Shargel, and A.B.C. Yu, *Applied Biopharmaceutics and Pharmacokinetics*, Appleton and Lange, Connecticut, 3rd Edition, 1993.

166. A.J. Jackson, and, M., Chen. Application of moment analysis in assessing rates of absorption for bioequivalency studies. *J. Pharm. Sci.* **76**(1):6-9 (1987).

167. K. Murata, and K. Kohno. Estimation problem due to multiple solutions in pharmacokinetic curve fitting to two-compartment model and its avoidance. *Biopharm. Drug Dispos.* **10**:15-24 (1989).

168. J.G. Wagner. Do you need a pharmacokinetic model, and,if so, which one? *J. Pharmacokin. Biopharm.* **3**(6):457-478 (1975).

169. J.G. Wagner. Linear pharmacokinetic models and vanishing exponential terms: implications in pharmacokinetics. *J. Pharmacokin. Biopharm.* **4**(5):395-425 (1976).

170. K.R. Godfrey, and M.J. Chapman, The problem of model indistinguishability in pharmacokinetics, *J. Pharmacokin. Biopharm.* **17**(2):229-267 (1989).

171. W.R. Gillespie. Noncompartmental versus compartmental modelling in clinical pharmacokinetics. *Clin. Pharmacokinet.* **20**(4):253-262 (1991).

172. M. Gibaldi, and D. Perrier. In J. Swarbrick (ed.), *Drugs and the Pharmaceutical Sciences: Pharmacokinetics*, **Volume 15**, Marcel Dekker, Inc., New York, 1982.

173. E.R. Garrett. Classical pharmacokinetics to the frontier. *J. Pharmacokin. Biopharm.* **1**(5):341-361 (1973).

174. H.J. Rogers, R.G. Spector, and J.R. Trounce, *A Textbook of Clinical Pharmacology*, Hodder and Stoughton Ltd., London, 1st Edition, 1981.

175. D.Z. D'Argenio. Optimal sampling times for pharmacokinetic experiments. *J. Pharmacokin. Biopharm.* **9**(6):739-756 (1981).

176. W.L. Chiou. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve. *J. Pharmacokin. Biopharm.* **6**(6):539-546 (1978).

177. K.C. Yeh, and K.C. Kwan. A comparison of numerical integrating algorithms by trapezoidal, lagrange, and spline approximation. *J. Pharmacokin. Biopharm.* **6**(1):79-98 (1978).

178. R.D. Purves. Bias and variance of extrapolated tails for area under the curve (AUC) and area under the moment curve (AUMC). *J. Pharmacokin. Biopharm.* **20**(5):501-510 (1992).

179. J.G. Wagner, and E. Nelson. Percent absorbed time plots derived from blood level and/or urinary excretion data. *J. Pharm. Sci.* **52**(6):610-611 (1963).

180. J.C.K. Loo, and S. Riegelman. New method for calculating the intrinsic absorption rate of drugs. J. Pharm. Sci. 57(6):918-928 (1968).

181. G.T. Tucker. The determination of an *in vivo* drug absorption rate. *Acta Pharm. Tech.* **29**(2):159-164 (1983).

182. L. Endrenyi, S. Fritsch, and W. Yan. Cmax/AUC is a clearer measure than Cmax for absorption rates in investigations of bioequivalence. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **29**(10):394-399 (1991).

183. J.K. Aronson, H.J. Dengler, L. Dettli, and F. Follath. Standardization of symbols in clinical pharmacology. *Eur. J. Clin. Pharmacol.* **35**:1-7 (1988).

184. S. Vožeh, O. Schmidlin, and W. Taeschner. Pharmacokinetic drug data. *Clin. Pharmacokin.* **15**(4):254-282 (1988).

185. M-L. Chen, and F.R. Pelsor. Half-life revisited: Implication in clinical trials and bioavailability/bioequivalence evaluation. *J. Pharm. Sci.* **80**(4):406-408 (1991).

186. M. Rowland, L.Z. Benet, and G.G. Graham. Clearance concepts in pharmacokinetics. *J. Pharm. Biopharm.* **1**(2):123-136 (1973).

187. D. Perrier, and M. Mayersohn. Noncompartmental determination of the steady-state volume of distribution for any mode of administration. *J. Pharm. Sci.* **71**(3):372-373 (1982).

188. K. Yamaoka, T. Nakagawa, and T. Uno. Statistical Moments in Pharmacokinetics. *J. Pharmacokin. Biopharm.* **6**(6):547-558 (1978).

189. D.J. Cutler. Theory of mean absorption time, an adjunct to conventional bioavailability studies. *J. Pharm. Pharmac.* **30**:476-478 (1978).

190. S. Riegelman, and P. Collier. The application of statistical moment theory to the evaluation of *in vivo* dissolution time and absorption time. *J. Pharmacokin. Biopharm.* **8**(5):509-534 (1980).

191. L.Z. Benet, and R.L. Galeazzi. Noncompartmental determination of the steady state volume of distribution. *J. Pharm. Sci.* **68**(8):1071-1074 (1979).

192. J.H. Oppenheimer, H.L. Schwartz, and M.I. Surks. Determination of common parameters of iodothyronine metabolism and distribution in man: noncompartmental analysis. *J. Clin. Endocrinol. Metab.* **41**:319-324 (1975).

193. Y. Kasuya, H. Hirayama, N. Kubota, and K.S. Pang. Interpretation and estimates of mean residence time with statistical moment theory. *Biopharm. Drug Dispos.* **8**:223-234 (1987).

194. P.S. Collier. Mean residence time: Some further considerations. *Biopharm. Drug Dispos.* **10**:443-451 (1989).

195. M.D. Karol. Mean residence time and the meaning of AUMC/AUC. *Biopharm. Drug Dispos.* **11**:179-181 (1990).

196. H.-U. Schulz, and V.W. Steinijans. Striving for standards in bioequivalence assessment: a review. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **30**(Suppl 1):S1-S6 (1992).

197. W.J. Jusko, and M. Gibaldi. Effect of change in elimination on various parameters of the two-compartment open model. *J. Pharm. Sci.* **61**(8):1270-1273 (1972).

198. S.M. Pond and T.N. Tozer. First-pass elimination: Basic concepts and clinical consequences. *Clin. Pharmacokin.* **9**(1):1-25 (1984).

199. E. Nelson. Kinetics of drug absorption, disposition, metabolism, and excretion. *J. Pharm. Sci.* **50**(3):181-192 (1961).

200. K.F. Ilett, L.B.G. Tee, P.T. Reeves, and R.F. Minchin. Metabolism of drugs and other xenobiotics in gut lumen and wall. *Pharmac. Ther.* **46**:67-93 (1990).

201. P.S. Collier, and S. Riegelman. Estimation of absolute bioavailability assuming steady state apparent volume of distribution remains constant. *J. Pharmacokin. Biopharm.* **11**(2):205-214 (1983).

202. C.M. Metzler, and D.L. Weiner. *PCNONLIN, Version 3.0, Software for the Statistical Analysis of Nonlinear Models on Micros.* Third Edition, Statistical Consultants, Inc., Lexington, Kentucky, U.S.A., 1989.

203. K. Yamaoka, T. Nakagawa, and T. Uno. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacokin. Biopharm.* **6**(2):165-175 (1978).

204. H. Akaike. An information criterion (AIC). *Math. Sci.* **14**(153):5-9 (1976).

205. K. Tanabe. Statistical approach for the unsuitable problem. *Math. Sci.* **14**(153):60-64 (1976).

206. W.J. Westlake. Problems associated with analysis of pharmacokinetic models. *J. Pharm. Sci.* **60**(6):882-885 (1971).

207. J.A. Clements, and L.F. Prescott. Data point weighting in pharmacokinetic analysis: intravenous paracetamol in man. *J. Pharm. Pharmac.* **28**:707-709 (1976).

208. P. Galtier, M. Alvinerie, J.L. Steimer, P. Francheteau, Y. Plusquellec, and G. Houin. Simultaneous pharmacokinetic modelling of drug and two metabolites: Application to albendazole in sheep. J. Pharm. Sci. 80(1):3-10 (1991).

209. *MIMS Desk Reference (MDR)*, **Volume 30**, Times Media Limited, Pretoria, 1994/95, p. 1438.

210. T. Walle, U.K. Walle, L.S. Olanoff, and E.C. Conradi. Partial metabolic clearances as determinants of the oral bioavailability of propranolol. *Br. J. Clin. Pharmacol.* **22**:317-323 (1986).

211. C.E. Inturrisi, W.A. Colburn, R.F. Kaiko, R.W. Houde, and K.M. Foley. Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. *Clin. Pharmacol. Ther.* **41**:392-401 (1987).

212. J.M. De-Vito, R.E. Crass, R.A. Blum, R.A. Pleasants, and J.J. Schentag. Estimation of steady state volume of distribution for digoxin: comparison of model independent methods with a two compartment model in healthy volunteers. *Drug Intell. Clin. Pharm.* **19**:837-839 (1985).

213. F.E.R. Simons, and K.J. Simons. H_1-receptor antagonists: Clinical pharmacology and use in allergic disease. *Ped. Clin. North Am.* **30**:899-914 (1983).